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Algae: Source to Treatment

MANUAL OF WATER SUPPLY PRACTICES — M57, First Edition

AWWA MANUAL M57
First Edition

American Water Works Association

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Algae: Source to Treatment

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Introduction

Jeff Janik

Finding solutions to drinking water problems caused by algae is an ongoing challenge to the water industry, from tastes and odors, to clogging of filters in water treatment, to harmful algal blooms of toxin-producing cyanobacterra. There is widespread belief that the frequency and severity of surface water impairment by algae is increasing, largely from human activities, leading to degradation of watersheds and increased eutrophication. The increase in taste and odor (T&O) events and cyanobacterial blooms have also been linked to drought and climate change. While the news is not all dire, there are many challenges facing those responsible for producing and delivering potable water. These new challenges necessitate an increased awareness, knowledge, and understanding by water professionals in dealing with problems caused by algae. Water professionals must be familiar with all aspects of solving algae-related problems, from identification of the problem-causing algal species, to design and implementation of monitoring programs using a larger array of tools, and finally, management and treatment strategies that are mindful of the overlying costs of these programs.

AWWA Manual M57, *Algae: Source to Treatment*, is a comprehensive collection written by experts in each respective subject area. The book is broadly divided into three main sections: Section I is devoted to methods of sampling and analysis; Section II provides a detailed overview discussion of the organisms, the major taxonomic groups of algae; and Section III consists of four chapters aimed at management of algae from taste and odor to toxins.

The four chapters in Section I provide guidance on methods for monitoring, sampling, and detecting algae.

New developments in online monitoring using Web-based technologies and real-time or near-real-time sensors useful in detection and monitoring of algae are addressed in Chapter 1. Specific instruments and their advantages and limitations are described, as well as case studies of monitoring programs that are currently operational. Suggestions are included on appropriate strategies for managing a program with the ultimate goal of tracking algal blooms and associated physical–chemical conditions in surface freshwater sources.

The process of obtaining useful qualitative and quantitative information about algal assemblages begins with the thoughtful planning of sampling objectives, knowledge of scientifically accepted sampling methods, and use of appropriate water sampling equipment. Chapter 2 provides guidance (especially for water utilities) on matching appropriate sampling strategies and equipment to the specific aquatic environments and algal communities being examined.

The water industry has two types of methodologies that can be used to detect cyanotoxins in potable water: screening assays and analytical methods for identification and quantification of cyanotoxins. In Chapter 3, sample preparation, screening assays, analytical methods, and genomic/proteomic techniques are reviewed. Methodologies for assessing the presence of these compounds are discussed, with recommendations on methodologies found effective by the authors' experiences and review of literature.

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Since chlorophyll *a* is present in both eukaryotic and prokaryotic algae, chlorophyll *a* concentration is most often used as a measurement of algal biomass. Accordingly, chlorophyll *a*–based assessments are useful in quantifying algal population/assemblage dynamics, physiological state, and growth rate in response to changing environmental variables. Likewise, this measure is also useful in comparing assemblage structure among systems and providing a basis for the understanding of cellular processes and energy cycling in algal assemblages.

Chapter 4 presents a synopsis of sampling and processing procedures, technological approaches, and general applications for the analytical methodologies most commonly utilized for algal chlorophyll measurement. For spectrophotometric, fluorometric, and chromatographic methodologies, the theoretical bases, history of methods development, and strengths and limitations associated with analytical protocols are presented.

In Section II, each of the nine chapters (5–13) is devoted to a major taxonomic group of algae. The biology, ecology, taxonomy, significance (to water supplies), taxonomic keys to identification of the common genera,* and glossary of terms are presented. For those seeking more information, all chapters are filled with extensive citations including recent literature. These chapters can serve as both an introduction to the algae for the nonspecialist and a source of detailed information for the specialist seeking the most up-to-date research.

The four chapters in Section III are devoted to management of algae including control and treatment strategies.

Chapter 14 considers source water assessment and protection from the perspective of noxious algal growth and treatment strategies for removing algal cells, toxins, and other undesirable bioactive substances. Examples are also presented of advanced technologies and approaches that are being applied to improve assessment, treatment (at potable water treatment plants), control (in surface source waters), and early warning systems for safeguarding water supplies from noxious algae.

Chapter 15 provides the background for understanding T&O production in algae. T&O compounds are produced by most algal groups though production of 2-methylisoborneol (MIB) and geosmin by cyanobacteria (blue-green algae) and are often the most problematic to water utilities. The T&O products in source water span the range from earthy, musty (MIB, geosmin), grassy, sulfurous, fishy/rancid, and cucumber-smelling to floral-smelling. A diversity of these volatile organic compounds is produced by a wide array of algal species. However, most T&O is caused by a relatively small number of these chemicals and taxa, and the majority of algal species have not yet been characterized relative to their T&O-causing abilities.

The focus of Chapter 16 is on strategies used for control of cyanotoxins. The appropriate strategy to be employed is dictated by a number of factors, including the cyanotoxin to be removed, whether the cyanotoxin is present within cyanobacteria cells or present outside of the cells, the cyanotoxin concentration in the source water, the target cyanotoxin concentration at the entry point to the distribution system, and technical capabilities of the control strategy.

Source water control of algae and a treatment plant's ability to remove algae from source water is an important step in delivering safe drinking water to the public. The focus of Chapter 17 is the removal of algae through drinking water treatment. Source water algae control and the removal of algal metabolites are discussed elsewhere in this text.

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* The key is a series of couplets with questions to answer while trying to make an identification. The number at right indicates where to go in the key next. For example, by answering yes to 2a, proceed to couplet 3, and by answering yes to 2b, proceed to couplet 16. Continue until you answer yes to a question that has a genus name next to it.

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Preface

The increased incidence of algae in drinking water supplies triggers a wide range of concerns for drinking water professionals. Our ability to mitigate biofouling, taste and odor, and toxin production issues depends on having a clear understanding of the organisms with respect to their biology, monitoring strategies, and treatment options. This is the inaugural edition of M57, *Algae: Source to Treatment*. This manual was written by a group of experts in their respective fields and provides background information regarding the most commonly found groups of algae. It provides practical information for identification of these organisms and their related toxins, along with strategies for reducing their occurrence and mitigating the harmful effects of these organisms when they do occur.

The Organisms in Water Committee of the American Water Works Association (AWWA) prepared this manual for water utility management, water quality, and public relations personnel to use as a reference tool. The manual provides scientific information in a concise, readable format that will be helpful to media, city and state government, and water customers who inquire about algae issues. Public health and environmental workers will also find it a convenient reference.

This manual includes chapters on the organisms, methods, and recommended management practices associated with algae. Each chapter has a reference section, and a detailed glossary is included at the end of the manual. Nine groups of organisms are described in detail. The organisms are grouped using older division names; however, each chapter provides taxonomic updates and references.

Regrettably, a Haptophyta chapter is not included in this edition because Paul Kugrens, the selected expert, unexpectedly passed away. Inclusion of specific information on haptophytes is planned for the second edition.

AWWA and the Organisms in Water Committee would appreciate any comments on the manual. Contact Elise Harrington, AWWA Environmental Engineer, or Molly Beach, Manuals Specialist, at (303) 794-7711, 6666 W. Quincy Avenue, Denver, CO 80235, or manuals@awwa.org with any feedback.

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Section I
Methods

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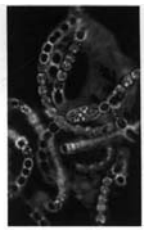

Chapter 1

Recent Developments in Online Monitoring Technology for Surveillance of Algal Blooms, Potential Toxicity, and Physical–Chemical Structure in Rivers, Reservoirs, and Lakes

Robert E. Reed, JoAnn M. Burkholder, and Elle H. Allen

SUMMARY

Disclaimer: The vendors, manufacturers, and equipment described in this work were chosen as representative examples of the types of instrumentation currently commercially available for use in the water-quality monitoring field. The inclusion of these examples does not constitute an endorsement by the Center for Applied Aquatic Ecology, North Carolina State, or AWWA. Sensor development and the area of online monitoring is changing rapidly, therefore individuals interested in specific monitoring areas or equipment types are encouraged to use an Internet Web search to determine all possibilities.

Previous AWWA manuals and review papers have discussed the application of many online monitoring tools (e.g., Grayman et al. 2001, Knappe et al. 2004, Glasgow

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et al. 2004), but in the last ca. five years significant advances have been made in communication technology and instrument development, resulting in the introduction of many new products that are useful in monitoring algae and associated water quality parameters. The main objective of this chapter is to introduce the reader to new developments in online monitoring using Web-based technologies and real-time or near-real-time sensors useful in the detection and monitoring of algae. Specific instruments and their limitations will be described, as well as case studies of monitoring programs that are currently operational. Suggestions are included of an appropriate strategy for managing a program with the ultimate goal to track algal blooms and associated physical–chemical conditions in surface freshwater sources.

A successful online monitoring program requires appropriate planning in the installation and deployment of online monitoring equipment, and in choosing methods for the acquisition and postprocessing of the data for efficient operational and management decisions. Large amounts of data are acquired by this type of program due to the short-time-scale sampling that is possible with presently available equipment. Moreover, efficient communication systems enable rapid uploads of data to the data acquisition computer (DAC) and Web site. The rapid development in this technology has led to availability of dedicated hardware and software through many commercial vendors. It is also possible for a water treatment plant or municipality to create custom-designed systems for specific problems using commercially available hardware and in-house information technology personnel. Initial investment in planning will yield an online system with functional, valuable data products to assist operators and managers.

Recent important developments in pigment sensor technology have enabled improved tracking of algal blooms in real time and provide an early warning system for water treatment plant operators and managers. Various manufacturers have integrated both chlorophyll (indicator of total phytoplankton biomass) and phycocyanin pigment sensors (the latter, a major pigment in cyanobacteria) into multiparameter water quality sondes, allowing remote assessments of relative fluorescence. These remote data inform interested parties of impending or existing changes in algal biomass in real time or near-real time. With this early warning, dedicated field samples can be taken to quantitatively assess biomass, species composition, cyanotoxins, taste and odor compounds, and other parameters of interest. Remotely acquired online data for algal pigments are in relative fluorescence units, however, and should be calibrated for each bloom event by dedicated discrete field sampling for chlorophyll *a* and phycocyanin concentrations, and through analysis by standardized laboratory methods.

Many options for instrumentation and modes of deployment are available for establishing a Web-based online monitoring system. Laboratory-based instruments include various flow-through devices that can be installed in-line with source water to give an accurate real-time assessment of algal blooms. In addition to standard packages for environmental conditions, these instruments include the above-mentioned pigment sensors. Recently, a quantitative biosensor also became commercially available, which uses bluegill sunfish as "sentinels" for toxic conditions in source waters. This technology senses not only the presence of harmful algal toxins, but also other substances that are toxic to fish. Instruments and systems available for field deployment include fixed-structure and buoy-based water-column profilers and autonomous underwater vehicles (AUVs) that can be programmed to assess water quality in large reservoirs.

The data acquired by profilers and in-line source water instruments are limited spatially since they are fixed site deployments, whereas data from AUV technology are limited temporally because of the high cost and effort involved to continually run AUVs. Online real-time monitoring systems need to be supported by both regularly scheduled and event-driven field sampling efforts to calibrate the instruments. In

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addition, remotely sensed pigment data require calibration with samples analyzed using standard laboratory methods. Overall, the online monitoring programs built around this equipment, in concert with standard Web-based information technology, can provide operators and managers with valuable early warning system capability for detecting and tracking harmful algal blooms in real or near-real time.

INTRODUCTION

Potable water treatment plants need to monitor source water conditions on a timescale as close to real time as possible. Such data provide managers and operators the capability to detect changing conditions, such as developing algal blooms or turbidity/pollutant spikes, that require immediate action for efficient, cost-effective water treatment. Increases in the nutrient pollution (cultural eutrophication) of surface waters can lead to algal blooms, the occurrence of taste and odor causing organisms, and potentially toxic algal species (here including cyanobacteria; e.g., Wetzel 2001, Touchette et al. 2007, Burkholder et al. 2010, Botana 2007). Anthropogenic impacts on reservoirs will continue to intensify in the future due to increases in population growth and watershed development. Algal blooms can develop rapidly at distant sites and can then be transported to municipal source water intakes by increased precipitation/flow, by changes in wind magnitude and direction, or from controlled water release from dams as examples. In order to sample and monitor the dynamic nature of algal blooms, source waters should be monitored, insofar as economically feasible, at both high temporal and small spatial scales.

The need for drinking water is on the rise due to urban development and population growth in many parts of the United States. At the same time, water shortages and usage restrictions are becoming more frequent due to shortages caused by climatic events, such as long-term droughts (Arnell 1999, Lettenmaier et al. 2004, Milley et al. 2005). The lower flushing rates imposed by droughts often promote algal blooms in nutrient-enriched (eutrophic) surface waters (Wetzel 2001). Many water plant operators and managers often do not have the lead time to sample small-scale events or the early warning notification to control problems such as increases in potentially toxic algal blooms. Thus, commonly warning of impending, in-progress, or recent increases in algal biomass is captured during field-based sampling of a water source by water plant personnel or following complaints from recreational users and other concerned citizens.

Traditional field efforts for water quality monitoring are cost and labor intensive due to the requirement of personnel, vehicles, and sampling gear, and can be augmented and strengthened using online and real-time-accessible equipment. Instruments such as multiparameter sondes are underutilized when monitoring programs depend on field sampling efforts exclusively. Infrequent sampling is conducted in most standard field monitoring programs, and this frequency misses many pollutant-loading events that occur on smaller timescales. New Web-based technologies and developments in real-time monitoring equipment, with hourly or more frequent sampling capability 24/7, can be valuable in the assessment of algal blooms and the deleterious changes in coupled physical–chemical conditions (e.g., increases in nutrients, turbidity, pH, and dissolved oxygen). The information gained from the detection of developing algal blooms and pollutant spikes in real or near-real time can be a powerful tool in guiding day-to-day decisions in water treatment protocols.

Recent introductions of commercial water quality monitoring equipment have included buoy- and piling-mounted water-column profilers useful in monitoring biological and physical–chemical parameters throughout the entire water column. Extended deployment water quality instruments equipped with antibiofouling measures have

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pigment-specific sensors that allow real-time monitoring of algal biomass as relative chlorophyll or phycocyanin fluorescence. AUV technology recently has been designed to assess water quality in entire reservoirs, providing three-dimensional representations of the reservoir environment. In addition, the recent application of biomonitoring technology using fish as biosensors has resulted in a commercially available system to monitor a wide range of chemicals and environmental stressors, including emerging and unknown toxicants in source waters. These significant new hardware developments have been accompanied by new water quality software management tools. The large amounts of data acquired by these technologies require user-friendly postprocessing and visualization software packages for rapid, accurate management decisions and public notification.

DEPLOYMENT AND OPERATIONAL CONSIDERATIONS IN ONLINE MONITORING PROGRAMS

The development of a sound strategy that considers the data of interest, the site or sites of interest, and the amount of funds dedicated to the effort is necessary for maximum success before initiating an online monitoring program. Depending on the number of monitoring units that are economically feasible for a given real-time program, the primary sensor should be placed at or near the intake to drinking water treatment plants to afford depth profiles in real-time assessment of physical–chemical conditions and algal abundance. Instruments can also be placed in-line with raw water intake pipes between the reservoir and the water treatment plant. Secondary sensors that are deployed should be located at sites distant from the intake where the majority of the upstream or incoming flow and pollutants can be assessed; for example, sensors mounted upstream from the intake on bridge pilings, situated in an area where the reservoir narrows and large volumes of water flow past. If a suitable hard structure such as on a bridge piling or channel marker is not available for mounting instruments, a floating platform or buoy system can be used. However, floating systems are often logistically hard to maintain and there are potential difficulties related to liability and in obtaining the proper permits for deployment. Problem areas in a reservoir known to have recurrent algal blooms or water quality problems are also candidates for real-time monitoring, such as where algal blooms historically occur or where there are extended water residence times or low flushing rates. The acquisition of online and real-/near-real-time field data can be programmed to occur at varied timescales depending on the user's requirements. Due to the stand-alone nature of the equipment, timescales of minutes to hours can be achieved, giving maximum temporal resolution in the data.

Large amounts of data can be acquired quickly in an online monitoring program, which can overwhelm a facility that has limited data acquisition, management, and decision-making power. It is important to acquire high-quality, properly calibrated data that can be viewed by managers and other users with short lead-time. It is also vital that programs with numerous field sites have the ability to manage, process, and present the large amounts of data in an easily interpretable format. To this end, a necessary feature of online monitoring programs is a robust data acquisition and postprocessing software package. Web-based technologies and software packages (e.g., MATLAB, LabView, Campbell Scientific RTMCPRO) are available that can seamlessly integrate data acquired in real time with user-specific programming based on individual needs. These software packages can be specifically configured for data acquisition, visualization, and dissemination via the Internet.

The construction of a user-friendly Web site consisting of graphics that are easily understood is also essential in online monitoring programs. Multilevel,

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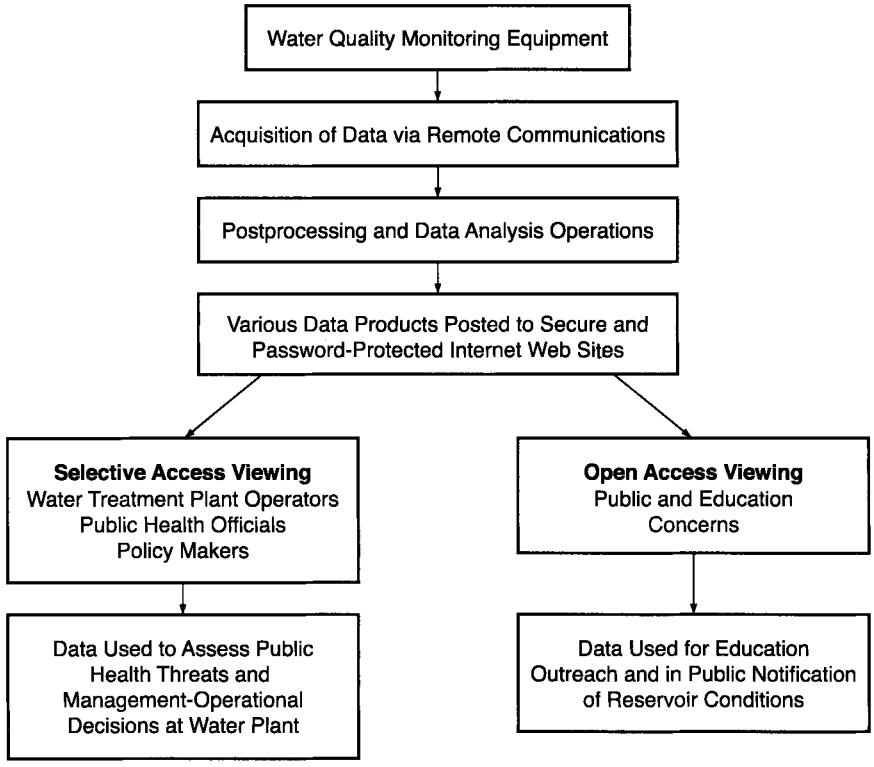

Note: After data acquisition and postprocessing, water quality data managers can post various data products to Web sites based on final use. Products used in water plant and public health management and operational uses can also be password-protected to allow access only by authorized personnel. The acquisition of high temporal- and spatial-scale data allows more confidence for managers and policy makers in decisions about appropriate water treatment protocols during algal blooms. Educational organizations and the general public can access the data for use in instructional and recreational activities, respectively.

Figure 1-1 A generalized flow diagram of data acquisition and product dissemination illustrating how water quality monitoring products can be distributed to both public and private concerns (Courtesy of the NC State University Center for Applied Aquatic Ecology)

password-protected sites on the same Web site are a popular method of providing data to various user groups. Web pages can be constructed to provide the public with information concerning water quality conditions and the presence of algal blooms or unusual physical–chemical conditions. More detailed Web pages, accessible via a password, can be constructed for a specific audience (Figure 1-1). Graphics and data sets addressing specific geographical locations or water plant operations can be made available to local utility managers and operators to assist in decision making. For instance, the presence of large numbers of taste and odor causing algal species can be communicated through a secure Web site, and appropriate actions can be taken by personnel responsible for water plant operations. Other important considerations in the acquisition and dissemination of algal and physical–chemical data are the duration between data acquisition and posting and in the validation and calibration of online/real-time data. Developers of online monitoring systems must decide the minimum timescale that is acceptable and then build the system accordingly. This decision will have bearing, as well, on the type of offsite communication technology selected for use. If the timescale of interest is

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minutes to hours, for example, RF modems (spread spectrum radio) or satellite methods of communication would provide appropriate support. If the timescale of interest is multiple hours or even days, then cell phone modems can be used. Also, depending on the length of a program and the data acquisition timescale, a large archive of data can be obtained that can then be mined at a later date for information such as seasonal changes and the response of the source water (e.g., reservoir) to event-driven inputs (e.g., turbidity spikes from rain events, algal blooms, other physical–chemical events). The long-time-series data provided by these monitoring efforts can be valuable in assessing trends in water quality parameters in concert with changing land use and climatic events, and the efficacy of management strategies to reduce loadings of nutrients and other pollutants of concern.

An example of an online field monitoring program that uses currently available commercial technology for real-time collection and rapid dissemination of algal and physical–chemical water column structure in a reservoir is the North Carolina State University (NCSU) Center for Applied Aquatic Ecology North Carolina Reservoirs Cyanobacteria Project, originally funded by the North Carolina Department of Health and Human Services and the US Centers for Disease Control and Prevention. Using data acquired from water quality sondes, profiler technology, and custom LabView programming, investigators can monitor the water column from depth profiles for six parameters taken in both local and distant reservoirs. The parameters are user-selectable and can be chosen based on need. Data are acquired from each station's sonde by a data logger and then communicated via cell phone modem to a central DAC where the information is automatically posted to a Web site (www .ncsu.edu/wq and www.ncsu.edu/wq/RTRM/dp14/ index.html) for viewing. Depth profiles of these data (e.g., Figure 1-2) at 3-hour intervals show hydrological conditions during spring and summer. The first set of profiles (Figure 1-2A–F), taken 24 April 2007, shows a spring phytoplankton bloom and the associated water-column conditions. The second set (Figure 1-2G–L) document the water-column structure during well-stratified summer conditions on 2 July 2007. These data are being used by local water plant managers to assess changes in source water quality in near-real time for modifying treatment protocols.

Commercial enterprises have recently developed data management services that include the acquisition of the data and/or data postprocessing and delivery of final data summaries including graphic visualizations. For example, EcoNet® (YSI Inc.) is an Internet-enabled data management system designed to collect and analyze water quality and hydro-meteorological data. This Web-enabled remote monitoring and control system is designed to acquire and postprocess large amounts of data that are then available via the Internet for user accessibility and management decisions. An example of such an operational system is the Mecklenburg County, N.C., Continuous Monitoring and Alert Notification Network (CMANN). This system includes 24 continuously sampling multiparameter water quality sondes linked to the Internet via EcoNet. The purpose of the CMANN program is to provide real-time water quality information to public works officials and other end users via specific Web sites when environmental changes occur on a short timescale.

PIGMENT SENSORS: FIELD MEASUREMENTS VERSUS LABORATORY METHODS

Fluorescence-based sensors are powerful tools for monitoring relative algal biomass, but care is needed in interpreting the fluorescence measurements with actual algal numbers or chlorophyll concentration data (Kirk 1994). These sensors can provide both discrete and continuous data concerning the presence of algae but are relative in comparison to

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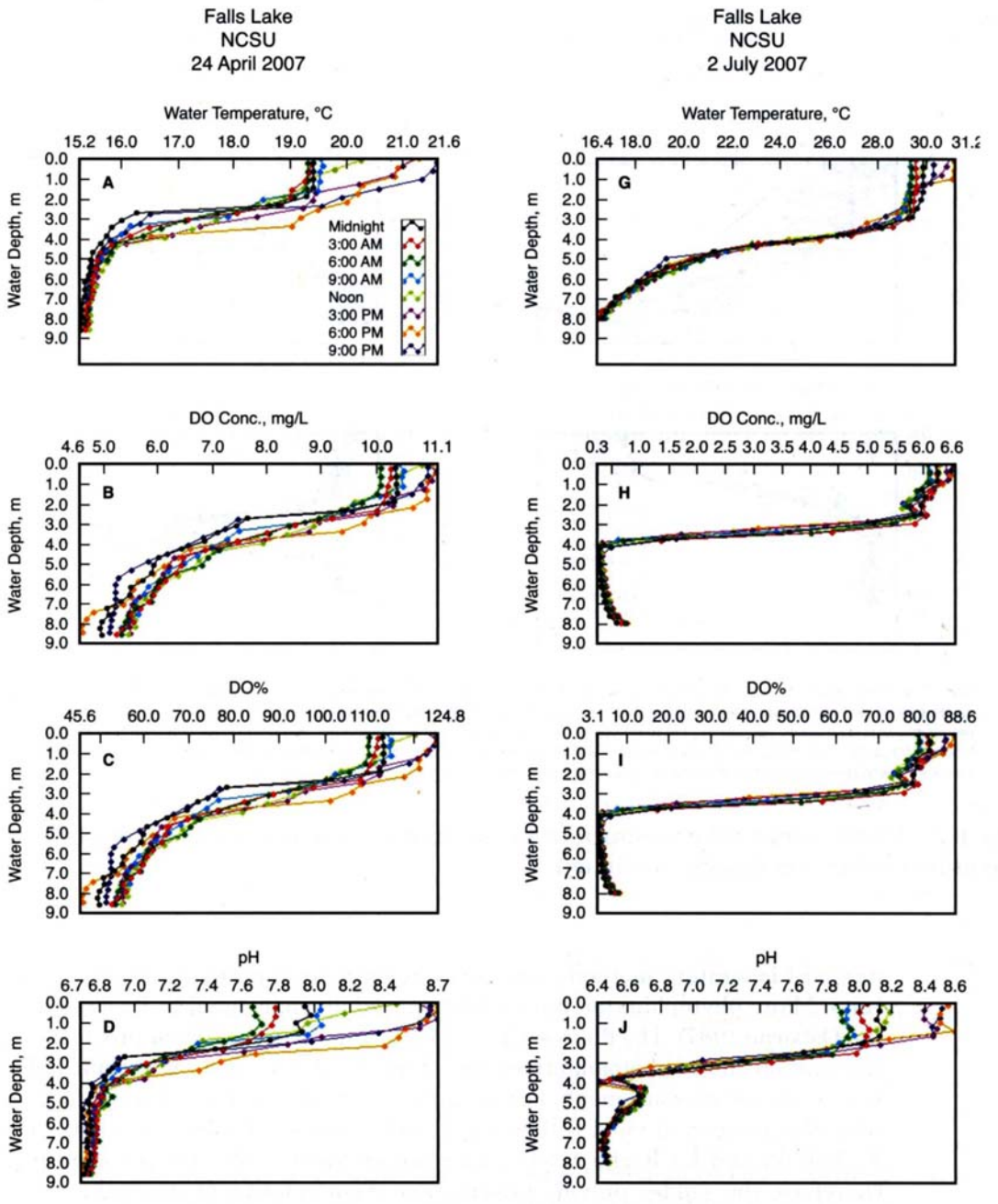

NOTE: The graphs illustrate two selected days, which include three hourly casts resulting in eight complete profiles per day. The first series (panels A–F) show water temperature, DO, pH, turbidity, and chlorophyll relative concentration on 24 April 2007. These plots reveal interesting structure concerning the thermocline, high pH values, turbidity structure, and chlorophyll structure due to a spring bloom. The second series (panels G–L) show the same parameters on 2 July 2007, revealing a strong thermocline, anoxic conditions below 4 m, and peaks in turbidity and chlorophyll structure just below the thermocline.

Figure 1-2 Whole water column structure of site located at a municipal water plant reservoir intake using profiler technology (Courtesy of NC State University Center for Applied Aquatic Ecology)

Figure continued next page

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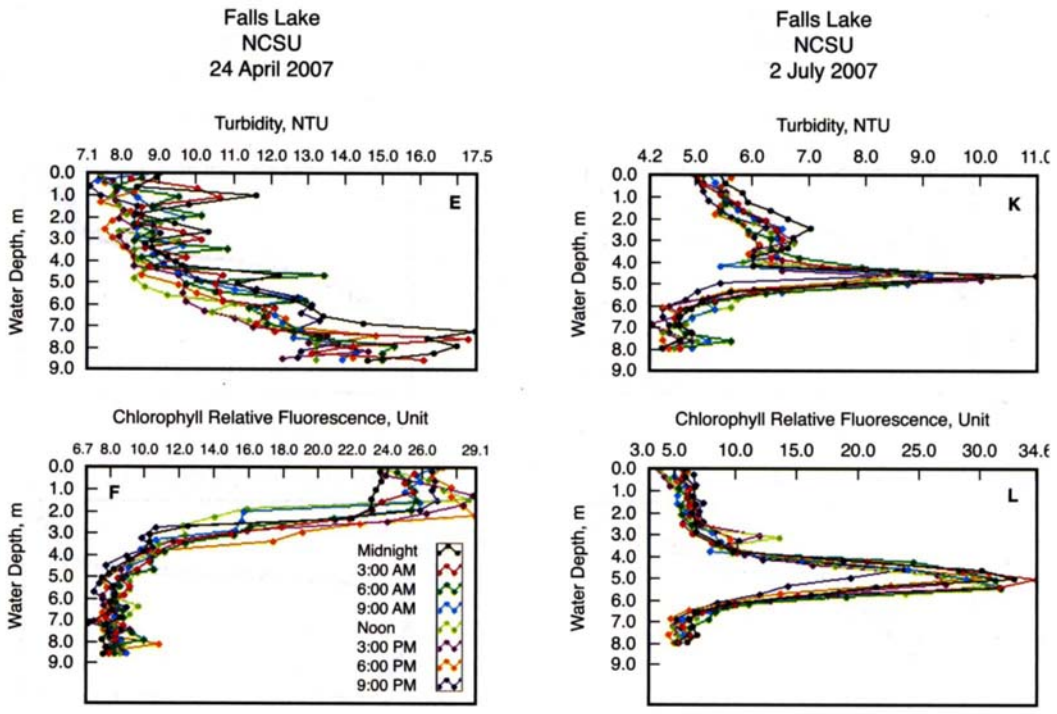

NOTE: The graphs illustrate two selected days, which include three hourly casts resulting in eight complete profiles per day. The first series (panels A–F) show water temperature, DO, pH, turbidity, and chlorophyll relative concentration on 24 April 2007. These plots reveal interesting structure concerning the thermocline, high pH values, turbidity structure, and chlorophyll structure due to a spring bloom. The second series (panels G–L) show the same parameters on 2 July 2007, revealing a strong thermocline, anoxic condition below 4 m, and peaks in turbidity and chlorophyll structure just below the thermocline.

Figure 1-2 Whole water column structure of site located at a municipal water plant reservoir intake using profiler technology (continued)

standard laboratory methods, and thus the data are reported as relative fluorescence units. Many phytoplankton have a pronounced diurnal signal in fluorescence (Geider and Osborne 1987). The fluorescence yield measured by the sensor provides "snapshot" information on the photoadaptive state of the algal cells, and the specific light regime, at the time of measurement. For example, if an algal cell is adapted to high irradiance, the fluorescent yield will be less than the same cell adapted to lower irradiance (Falkowski and La Roche 1991). Fluorescence yield is also temperature dependent. Therefore, the sondes provide information about relative phytoplankton abundance but not quantitative data on algal biomass or chlorophyll concentrations. For specific blooms of interest quantitative data on algal biomass (biovolume) can be obtained for comparison using standard light microscopy techniques (e.g., Lund et al. 1958, Burkholder 1992, Wetzel and Likens 2000). Chlorophyll concentration data can be obtained using standard laboratory instruments (fluorometers, spectrophotometers, high-performance liquid chromatography [HPLC]) after extraction in organic solvents (USEPA 1997, method 445.0; APHA et al. 2005, standard method 10200 H; Schlüter et al. 2006).

Recommendations from researchers and sensor manufacturers are to calibrate the relative fluorescence measured by the instrument to a known biological entity for each specific bloom of interest; for example, a unialgal culture can be used in the case of monospecific blooms, or a known concentration of an extracted pigment (e.g.,

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chlorophyll *a* or phycocyanin) can be used for mixed blooms. Highly purified algal pigments can be purchased from various chemical suppliers and used to standardize sensor readings to a known pigment concentration. The same calibration factor should not be relied upon from bloom to bloom because of the factors previously mentioned. The best comparative approach for a specific bloom of interest is to collect several grab samples in the field concomitantly with fluorescent sensor measurements and then determine the pigment concentration of the grab samples using standard laboratory extractive methods. The pigment concentration measurements can then be used to "normalize" the field fluorescence sensor data.

MULTIPARAMETER WATER QUALITY SONDES AND PIGMENT-SPECIFIC SENSORS

If one type of instrument is to be recommended as the standard instrument for water quality monitoring in both field and water treatment plant monitoring efforts, it is the multiparameter water quality sonde (the most common form factor is shown in Figure 1-3). These instruments are manufactured by many companies, for example, YSI, Hach-Hydrolab Inc., Turner Designs Inc., Greenspan Inc., In Situ Inc., and Eureka Environmental Engineering. The first three of these vendors manufacture multiparameter sondes that are equipped with pigment-specific sensors (i.e., for freshwaters, chlorophyll *a*, and phycocyanin) along with standard sensors such as temperature, dissolved oxygen (DO), pH, and oxidation reduction potential. These instruments are equipped with a sensor head area and a body that contains the microprocessor, which is responsible for data acquisition and computational functions necessary for calibration and communication. It should be noted that various vendors specialize in optical measurements of ocean water, including devices to measure chlorophyll, phycocyanin, and phycoerythrin (two common cyanobacterial pigments), but these companies are not active in equipment manufacture for water plant operations so they are not considered here.

Recent developments in sensor technology and other mechanical improvements have increased the utility of the multiparameter sonde, hereafter referred to only as "sonde." Two primary improvements are the use of luminescent detection technology to design improved pigment-specific sensors and the use of DO sensors. Chlorophyll *a*- and phycocyanin-specific sensors are presently available from YSI, Hach-Hydrolab, and Turner Designs; luminescence-based DO sensors are available from YSI and Hach-Hydrolab. This new technology replaces the standard Clark type and pulsed electrodes that were previously used on sondes to measure DO. Increases in DO and pH values are often indicative of an algal bloom; therefore, the data can often be used as a proxy for the occurrence of algal blooms when a fluorescence-based sensor is not available. The distinct advantages in use of luminescent DO sensors are (1) no membrane or electrode solution to replace and (2) longer deployments between calibrations. Another development in sonde technology is the use of automated wipers to clean biofouling organisms that grow on sensor surfaces. These wipers can be programmed to clean the sensor on a regular basis, thereby allowing greater accuracy in field measurements and longer intervals between maintenance.

A primary advantage in using sondes is that they can be set up for automatic data acquisition using industry standard data loggers and can also be deployed unattended where data is logged to an internal memory. Most sondes are configured to communicate with data loggers using standard SDI-12 and RS-232 protocols. A program resident in the data logger queries the sonde for data, which is then logged to a data logger. The data can be accessed via various communication methods such as cell phone,

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Figure 1-3 Close-up photographs of six commercially available water quality sondes equipped with multiple sensors and antifouling wipers: (A) YSI 6600V2 (Courtesy of R. Ellison, YSI, with permission); (B) Turner Designs C6 (Courtesy of Turner Designs, with permission); (C) Troll (Courtesy of In Situ); (D) CS304 sonde (Courtesy of Greenspan); (E) Hach-Hydrolab DS5x (Courtesy of R. Reed); (F) Manta (Courtesy of Eureka Environmental). These instruments can be controlled using standard industry data loggers in either the SDI-12 or RS232 modes, for data acquisition, postprocessing, and posting to Internet sites for user viewing.

satellite, and spread spectrum RF radio modems after the data have been recorded, postprocessed, and posted to a Web site for viewing. These multiparameter systems can be deployed in reservoirs distant from a water treatment plant (e.g., upstream) to provide warning of incoming algal blooms. They can also be configured to acquire data automatically at a specific time interval while sampling water pumped onboard or towed behind a boat, resulting in high resolution data. A detailed time series of test

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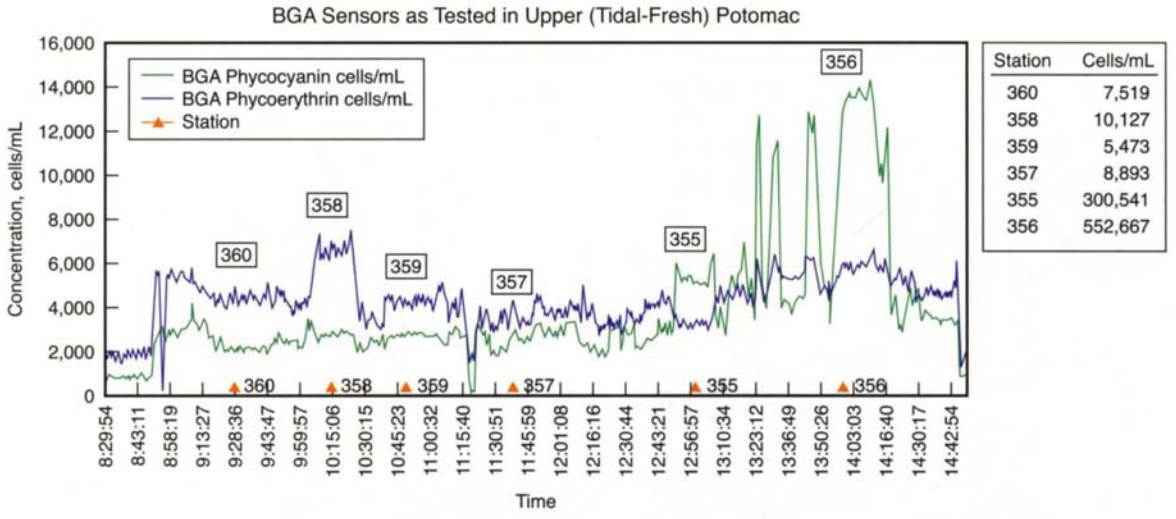

NOTE: The white-boxed numbers indicate the station numbers. The sensors were compared to evaluate whether the PC or the PE sensor was more effective in this estuarine area, which is known for extensive *Microcystis* blooms. The PC sensor provided better response to cyanobacterial biomass. Cell count data (via light microscopy) from samples taken at each station are indicated on the right side of the chart. The PC data correlate well with the cell counts. This time series illustrates the patchy nature of cyanobacteria biomass along the transect.

Figure 1-4 A time series of data acquired in the upper Potomac (brackish waters) using YSI phycocyanin (PC) and phycoerythrin (PE) sensors (Note: PE detectors are not available for freshwaters). (Courtesy of R. Ellison, YSI, with permission.)

data acquired in the upper Potomac (Figure 1-4) illustrates the variability in cyanobacteria biomass over long distances. This information is useful in assessing the present condition of source water in reference to the occurrence and location of algal blooms. These instruments can also be deployed at water intake structures. They are small and can be moved to other areas on short notice if deemed necessary. Readers are encouraged to check sonde manufacturers' Web sites for new technological developments and products regarding these instruments.

In order to ensure the acquisition of valid data that can be relied on for management, planning, and research efforts, a rigorous quality assurance/quality control protocol must be in place and documented with a quality assurance project plan. It is suggested that individuals involved in environmental monitoring and technology visit the USEPA Web site at www.epa.gov/quality for up-to-date information on quality management tools and other guidance specific to the proper acquisition of environmental data. One example of the need for detailed and accurate record keeping is in the case of sensors deployed under harsh field conditions where it is important to maintain individual calibration and maintenance logs for each sensor. For example, initial values of the sensors should be recorded before and after calibration to give a record of any sensor drift. This data is also useful as an early warning to sensor failure, allowing the technician to be proactive in the replacement of equipment. Manufacturer recommendations should be strictly adhered to at a minimum concerning calibration and maintenance; however, site-specific conditions will oftentimes require a more vigorous approach to equipment maintenance and calibration due to biofouling. Many manufacturers of water quality sondes have introduced self-cleaning features that remove biofouling films with wipers (e.g., YSI, Turner Designs, Hach-Hydrolab). Systems using older equipment or equipment not equipped with self-cleaning features must be rigorous in calibration and maintenance to avoid erroneous data due to biofouling, especially in highly eutrophic systems and during the warm seasons.

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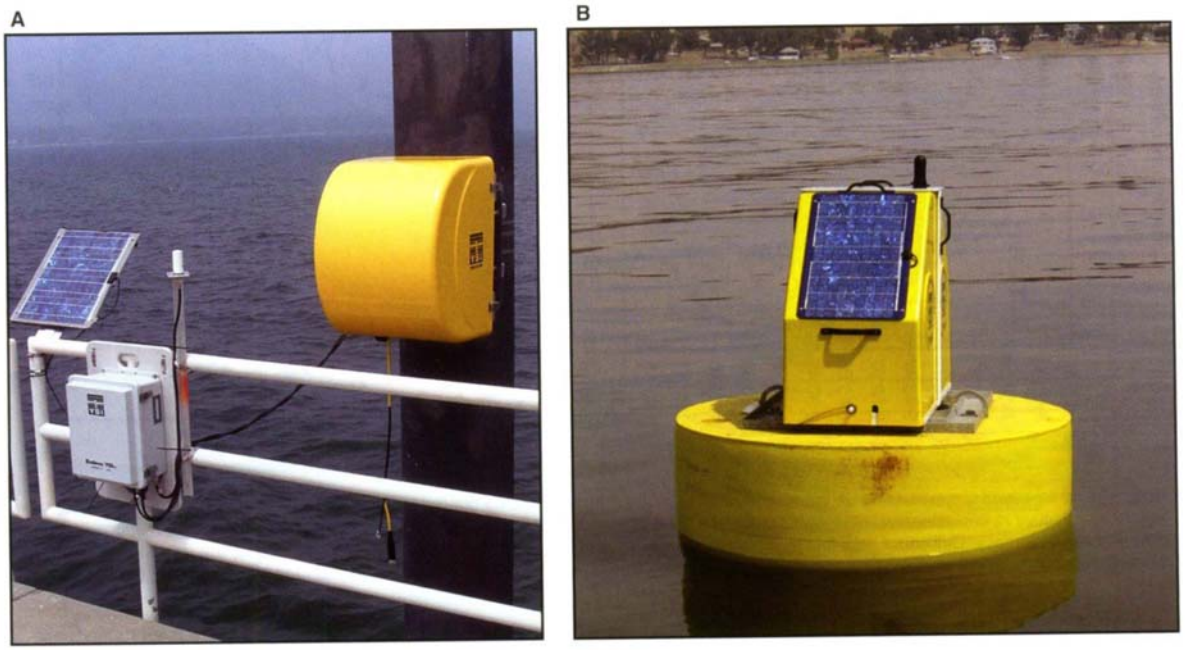

Figure 1-5 Two commercially available water-column profilers. (A) The fixed structure-mounted version is designed to be mounted on hard structures such as water intakes, piers, or bridge pilings. (B) The buoy-mounted profiler version is deployable in open water using two-point mooring. Both units are accessible via commercially available communications devices such as RF modem, cell phone, and satellite. The fixed structure-mounted instrument is also accessible via landline phone. (Courtesy of R. Ellison, YSI, with permission.)

Flow-through systems installed in-line to raw water intake pipes should also be inspected and cleaned regularly to remove any biofouling or foreign material.

EXAMPLES OF WATER-COLUMN MONITORING: PROFILING PLATFORMS, AUV TECHNOLOGY, AND BUOY-BASED SYSTEMS

Buoy and fixed-structure water quality profiling systems, which monitor the entire water column, recently have become commercially available. These systems can monitor depth-dependent structures for issues including turbidity spikes, chlorophyll spikes, high and low DO, pH variability, and phycocyanin spikes, providing valuable information for water plant operators in selecting appropriate filtration and other treatment protocols. If the water plant has a raw-water intake structure that allows water to be pulled from various depths, the data can inform plant operators of undesirable, depth-dependent changes in water quality that can then be avoided by taking water from a different depth. Examples of currently available commercial equipment are the YSI model 6952 buoy-based vertical profiling system and the model 6950 fixed system (Figure 1-5). The latter system is based on technology developed by scientists at the NCSU Center for Applied Aquatic Ecology (US Patent 7,040,157). A water quality sonde along with a computer-controlled winch allow acquisition of water-column biological and physical–chemical data at user-selected intervals. The data are communicated from the remote site to the water treatment facility using various commercially available devices (cell phone modems, spread spectrum RF modems, satellite, and so

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forth). A pontoon-mounted system (model 6951), similar to the buoy-mounted form factor, is also available for use in quiescent waters.

Examples of water-column data acquired by a fixed mount system are shown in Figure 1-2 (also see Deployment and Operational Considerations in Online Monitoring Programs). Contour plots of all water-column profiles acquired for a complete year at a municipal water plant reservoir intake using profiler technology illustrate the detailed biological, physical, and chemical structure that can be documented (Figure 1-6). Both longer-term (seasonal) and small-time-scale events can be recognized with multiyear or continuous profiler deployments, allowing managers the ability to be proactive in decision making, especially regarding problems that arise on a seasonal basis. The massive database acquired can also be useful in modeling efforts to improve predictive capability.

The advantages and disadvantages should be considered when selecting which system to deploy, especially site-specific characteristics of the reservoir. The fixed system can be mounted on water intake structures, piers, and bridge pilings and will be more stable from high wave action due to wind events. Moreover, if the system is mounted on a readily accessible water intake structure, operators will be able to maintain and calibrate the system easily. Buoy-mounted systems are less stable in high wind and wave conditions, but can be relocated to areas of interest by towing with a boat. On the other hand, a boat is required for maintenance and calibration of this system.

Monitoring large areas in a source water, or the entire source water, has been problematic because of the amount of man-hours and equipment that are required. The recent advent of commercially available AUV technology enables source water quality monitoring in three dimensions, permitting an assessment to be performed on the entire water body with limited personnel. The YSI EcoMapper AUV (Figure 1-7) is designed to be launched by one person; it autonomously surveys a water body using preprogrammed course and depth values. The AUV is fitted with standard sensors to allow the same multiparameter data to be acquired as with water quality sondes. The vehicle can measure a maximum of 10 water quality parameters at one-second intervals during missions up to 12 hours in length. Examples of turbidity and depth data acquired with this system are shown in Figures 1-7C and 1-7D, respectively. The high variability in the data, captured by the AUV, demonstrates the need for such high temporal and spatial sampling to provide operators and managers an overall assessment of depth-dependent water quality conditions, for example, near a potable water treatment plant, over small timescales. This system would be especially valuable in source waters where motorized boat traffic is not allowed, such as many restricted-access municipal reservoirs and recreational lakes. On the other hand, autonomous deployment of this equipment in source waters with high recreational use could be deleterious both to the equipment and to the general public, due to the probability of encounter with boat traffic.

Campbell Scientific Inc., Logan, Utah, markets a series of water quality monitoring buoys that can be used in freshwater ponds and small lakes with minimal wave action. These buoys are suitable for monitoring at a single fixed depth and do not have profiling capacity. The CSBUOY-DT and CSBUOY-NS buoys are self-contained packages that offer managers the ability to monitor water quality parameters via wireless technology. The effective range of these buoys is approximately ~1.6 km (1 mi) using a 7-A·h, 12-Vdc battery recharged with a 5-watt solar panel. Options are available to increase range to more than 16 km using a higher gain antenna with clear line of sight. After data acquisition and postprocessing with dedicated software, visualization software packages (e.g., LabView, MATLAB, Campbell RTMCPRO, or Vista Data Vision VDVSE) can be used to post the data from these platforms to Web sites.

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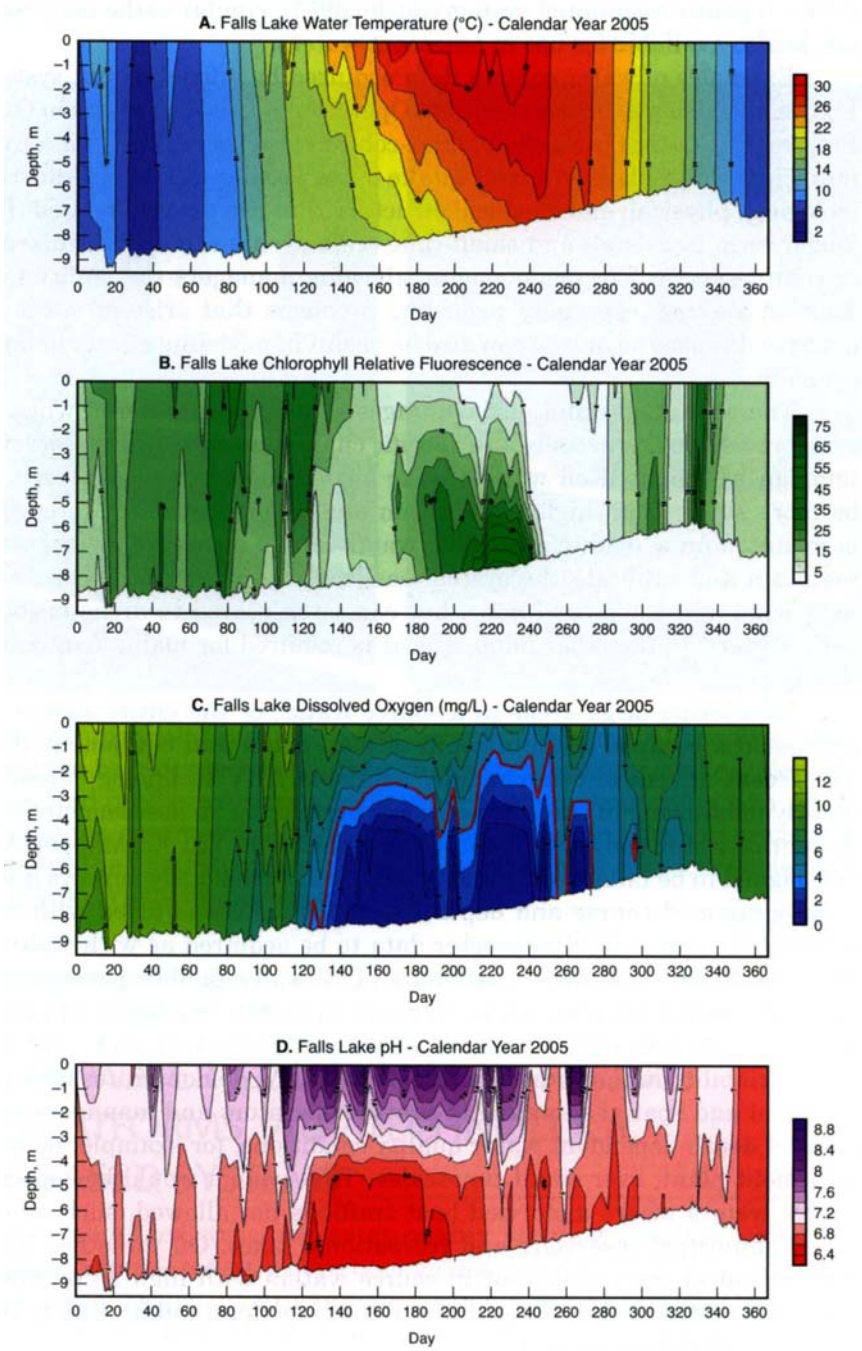

Figure 1-6 Contour plots, constructed using Surfer (version 7.0), of water-column profiles acquired at a municipal water plant reservoir intake using profiler technology during 2005, showing strong temporal and spatial resolution in all parameters: (A) Temperature. (B) Chlorophyll relative fluorescence; note spring phytoplankton blooms indicated at days ~80 and 120, and fall blooms at days ~320–330. (C) Dissolved oxygen, showing stratified DO conditions throughout the summer with low seasonal DO conditions from days 140 to ~260, followed by tropical storms that resulted in a well-mixed water column. (D) pH, again showing seasonally stratified conditions. Seasonal trends are clear in all parameters, as well as shorter-timescale signals in DO, pH, and chlorophyll relative fluorescence. These plots are useful to managers, water treatment plant operators, policy makers, and other end users in visualizing and presenting large amounts of data. (Courtesy of NC State University Center for Applied Aquatic Ecology.)

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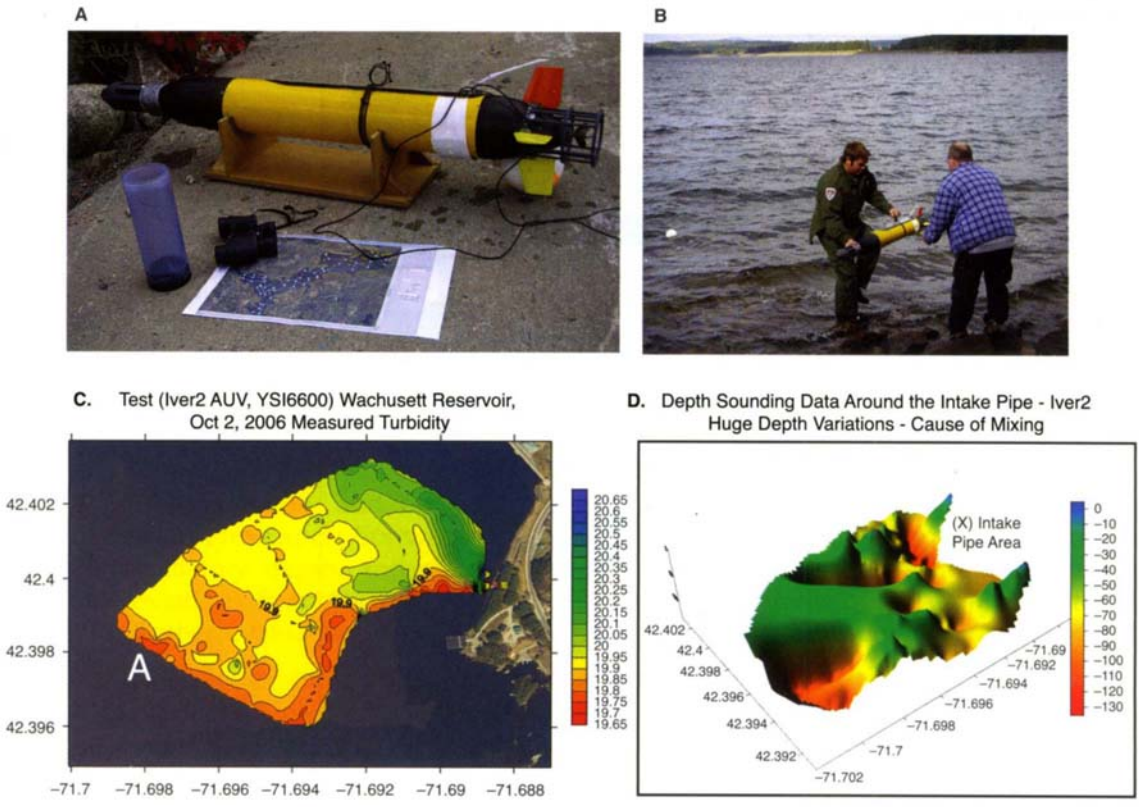

Figure 1-7 Illustration of YSI AUV available for monitoring reservoirs in three dimensions and associated data: (A, B) AUV is self-contained and is equipped with standard water quality sensors currently available, which can be seen at the tip of the AUV. The unit is small, which enables easy deployment and recovery. (C, D) Examples of turbidity and depth data acquired by AUV (Iver2, YSI6600) at the Wachusett Reservoir, illustrating the small-scale spatial variability of both parameters. (Courtesy of R. Ellison, YSI, with permission.)

ONLINE INSTRUMENTS FOR CHARACTERIZING SOURCE WATERS

The advent of online fluorescent monitoring equipment in recent years has provided early warning of the onset of blooms of taste and odor causing and/or toxic algae. It also enables background monitoring of algal biomass through daily and seasonal changes. Two variants of similar technology are offered by Turner Designs (Sunnyvale, Calif.) to monitor chlorophyll *a* and phycocyanin in source water. The AlgaeWatch™ and CyanoWatch™ instruments are based on fluorescence detection systems (relative fluorescence units), which Turner Designs has engineered and manufactured since 1972. AlgaeWatch monitors chlorophyll *a*, and CyanoWatch is designed to monitor phycocyanin, which can be used, along with simple confirmation by light microscopy, to detect cyanobacteria (Figure 1-8). Both instruments are designed to be installed in-line with source water pipes. The use of these sensors can assist management decisions on the need for various filtration and other treatment processes. These systems can be remotely accessed via a 4–20 mA output to a data logger, with dedicated software that allows 24-hour access to data via a Web-based computer.

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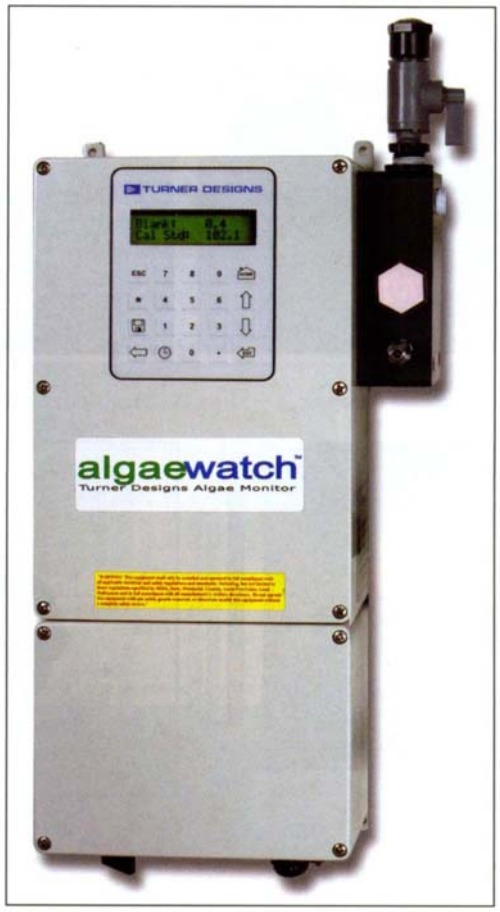

Figure 1-8 The Turner Designs AlgaeWatch online fluorometer. This instrument was installed by a municipal potable water treatment plant to sample source water for algal blooms. (Courtesy of Turner Designs, with permission.)

The use of fish as biomonitors for water quality is widespread in various parts of the world where water treatment plants use fish "sentinels" in tanks and other devices to assess changes in behavior due to stress from toxic substances and low water quality (Grayman et al. 2001). Concerns over water security have risen in recent years and have led to an increased interest in technology that is amenable to rapid toxic detection for water supplies (van der Schalie et al. 2001a, 2006). Research by investigators at the US Army Center for Environmental Health and Research has resulted in a commercially available product (US Patent 6,058,763) that monitors specific fish behavior parameters directly related to stress and toxicity. The device, called the intelligent Aquatic BioMonitoring System®, or iABS (Figure 1-9A), is currently marketed by Intelligent Automation Corporation (www.iac-online.com). This innovative aquatic biomonitoring system uses fish to assess toxicity of water supplies and source waters in a controlled and quantitative manner (Mikol et al. 2007, Shedd et al. 2001). The system is automated and includes exposure chambers that each hold a bluegill sunfish (*Lepomis macrochirus* Rafinesque). Body movements and ventilatory behavior (Figure 1-9B) are sensed by electrodes, recorded, postprocessed using the dedicated software Biomonitor Expert for visualization, and posted to a Web site. These data are easily viewed via the Web, and the software system can be configured to issue alarms triggered

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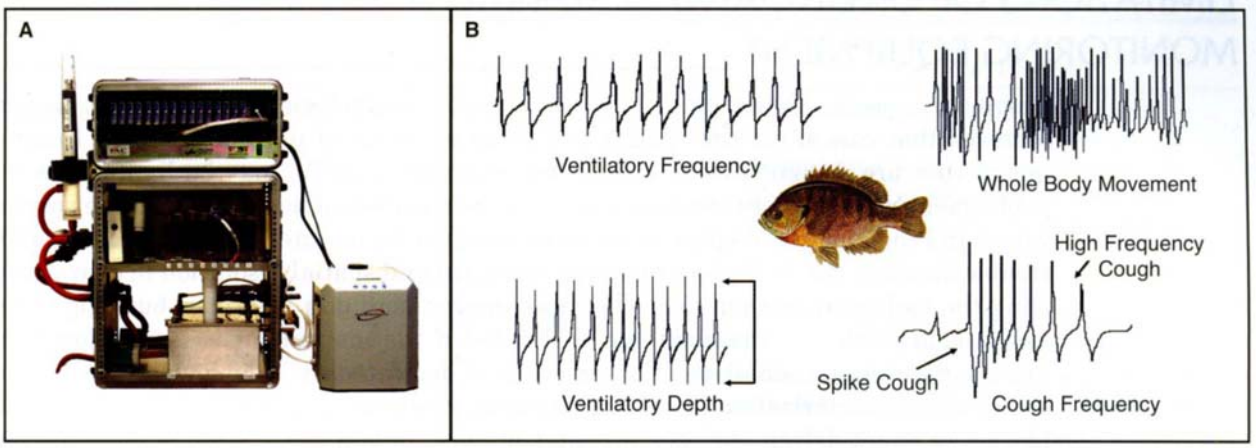

Figure 1-9 Fish ventilatory and behavior patterns monitored using the (A) IAC 1090 Fish Biomonitor system and Expert Software, which evaluates (B) ventilatory frequency, whole body movement, ventilatory depth, and cough frequency to determine the health and stress state of the fish. Deviations from baseline values result in an alarm and notification to water plant managers for further evaluation. (Courtesy of B. Lawler, Intelligent Automation Corporation Inc., with permission.)

by fish stress from toxic substances. This system has been tested successfully with some harmful algal species (van der Schalie et al. 2001b), illustrating its potential to provide early warning of toxic substances such as cyanotoxins in source water protection.

Other commercially available online biomonitoring equipment that uses biological entities to serve as sensors for water quality problems includes the Daphnia Toximeter, Fish Toximeter, and Algae Toximeter manufactured by bbe Moldaenke (www.bbe-moldaenke.de). These instruments are engineered such that the organism is exposed to a sample water stream and is monitored by motion analysis software in the case of the Fish and Daphnia Toximeters or software that records changes in fluorescence, in the case of the Algae Toximeter. Changes in organism response is recorded and interpreted by the dedicated software to trigger alarms or indicate changes caused by toxic or deleterious conditions, providing water system operators early warning to potentially hazardous events. The AlgaeOnlineAnalyser, also from bbe Moldaenke, continuously measures in real time chlorophyll fluorescence, algae class, and photosynthetic activity of natural algal assemblages in sample stream water. This instrument is useful in monitoring changes in algal communities and physiology that may occur over long periods of time due to various environmental impacts.

It should also be noted that existing instrumentation in water treatment plants can be integrated into an online monitoring program to assess changes in water quality due to algal blooms. For example, turbidimeters and particle counters from various vendors (e.g., HF Scientific Inc. [www.hfscientific.com] and Hach Inc. [www.hach.com]—turbidimeters; Hach—particle counters) use a variety of communication protocols (RS232, RS484, modbus, etc.), that allow maximum flexibility in online system integration. Increasing trends and spikes in turbidity and particle numbers are valuable information that can warn water plant operators as to changing conditions in raw water supply.

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LIMITATIONS OF ONLINE AND REAL-TIME MONITORING EQUIPMENT

There are specific limitations to all monitoring technologies due to basic design features that can affect the spatial and temporal scale of data acquisition. Instruments that are designed to be mobile, for example an AUV, may be limited due to deployment-recovery considerations such as the duration of deployment in the source water and the amount of effort that can be invested for maintenance, calibration, and troubleshooting. An AUV gives excellent temporal and spatial resolution in characterizing the biological and physical–chemical environment of a reservoir, but long-term assessment of the environment can be limited if the instrument is not deployed to provide a continuous database. The timescale of dedicated AUV deployments will constrain the characterization of rapidly changing conditions, such as initiation of algal blooms or event-driven changes in water quality, unless the system is operated on short timescales that allow maximum resolution.

Data acquired by sondes deployed at a single fixed depth, and profiling devices that are located at a single site obviously will be limited to characterization of that site and will miss environmental changes at distant sites. In the case of sondes deployed at a fixed depth, the user will not gain information on water-column conditions above or below the sonde. Another limitation in acquiring chlorophyll or phycocyanin fluorescent data using sondes and profilers occurs when trying to assess the abundance of algae that form benthic or floating mats, for example the potentially toxic cyanobacterium *Plectonema wollei* Farlow ex Goment (previously known as *Lyngbya wollei*).

Lakes and rivers are dynamic systems that can change rapidly because of variability in flow due to both anthropogenic and natural occurrences. Acoustic Doppler current profiler (ADCP or ADP) technology using either fixed and/or boat-mounted instruments can be used to monitor flow patterns important in tracking algal blooms and other water quality conditions (e.g., turbidity events and spills). Many commercial products are available and can be found with a general Web search using keywords such as ADCP, ADP, ADV, acoustic Doppler current profiler, or current meters.

FIELD METHODS USED IN CONJUNCTION WITH ONLINE MONITORING

There are many published manuals and textbooks with information and methods that describe basic considerations and methodologies for sampling source waters (e.g., APHA et al. 2005, Wetzel and Likens 2000). Field methods used for collecting algal samples and associated toxins must be adequate to enable further identification, molecular, and toxicity testing by water plant or supporting laboratories. In characterizing source waters, monitoring sites should be chosen to maximize the user's ability to characterize incoming pollutant sources, including known "hot spots" for algal blooms, fish kills, and DO deficits, as well as waters with the highest probability of human contact through recreational use (Meriluoto and Codd 2005). Real-time data should be groundtruthed by additional field sampling for parameters of interest such as phytoplankton biomass and dominant species, nutrients, and other pollutants.

Dedicated field sampling and associated analytical methods are useful in determining areas of high bloom activity and in identifying potentially harmful algae and their toxins. Commercially available toxin kits are available for some algal toxins and may be either quantitative or qualitative depending on the method and manufacturer (examples given in Table 1-1). Use of these kits can help water utilities assess the condition and toxin concentrations of source water and also the efficacy of filtration in the

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Table 1-1 Selected commercially available kits for cyanotoxin detection/quantification

Toxin	Product Number	Vendor	Description of Product
Microcystin	EP 022	Envirologix	ELISA Microtiter® plate kit for quantitative detection of microcystins in surface waters. Limit of detection, 0.147 ppb microcystin-LR equivalents. Limit of detection for the high-sensitivity alternate protocol (potable water), 0.03 ppb assay range 0.16–2.5 ppb.
	ET 022	Envirologix	QualiTube kit for microcystin, ELISA tube field kit for qualitative determination of presence/absence of microcystin in surface waters (limit of detection 0.3 ppb); tubes read visually or with a photometer; assay range 0.5–3.0 ppb.
	520011	Abraxis, LLC	Microcystins/Nodularins (Adda), ELISA kit, Microtiter plate. Limit of detection 0.10 ppb (assay range 0.15–5 ppb).
	520012	Abraxis, LLC	Microcystin tube kit; quantitative, requires photometer.
	520012F	Abraxis, LLC	Microcystin tube kit; qualitative, requires photometer.
	CPP-023	Beacon Analytical Systems Inc.	ELISA Microcystin plate kit. Limit of detection ~0.05 ppb.
Cylindrospermopsin	522011	Abraxis, LLC	Cylindrospermopsin ELISA kit, Microtiter plate for quantitative detection; limit of detection 0.04 ppb, assay range 0.05–2.0 ppb.
Saxitoxin	52255B	Abraxis, LLC	Saxitoxin ELISA kit, Product No. 52255B: Microtiter plate, quantitative determination, limit of detection 0.015 ng/mL, assay range 0.02–0.4 ppb.

final product. It is important, however, to cross-confirm enzyme-linked immunosorbent assays (ELISAs) and protein phosphatase inhibition assays with standard HPLC, liquid chromatography/mass spectroscopy (LC/MS), and other methods as appropriate (Tillmanns et al. 2007). Advanced LC/MS/MS methods may be used for toxin variant determination (Meriluoto and Codd 2005).

All sampling devices and containers must be free of any biological or inorganic residue before sample collection. Standard washing and acid stripping of glassware is necessary, along with the use of a cleaner that removes DNA such as DNA Away by Molecular Bio-Products or DNase Displace from Fisher Scientific. These precautions are especially important in the collection of material for DNA, nutrient, and algal toxin analyses to eliminate the possibility of contamination. It is also recommended that all sampling devices be rinsed twice with source water from the site prior to collection.

Most standard methods for determining algal toxins describe collecting the algal material from water samples or surface scums and then extracting toxins from algal cells collected by filtration or lyophilized bloom material. Samples should be processed and analyzed as soon as possible within 24 hours after collection to minimize degradation of toxin and algal material and to maximize recovery. If samples are to be sent to commercial laboratories for toxin analysis, they should be prepared the same day of collection according to the receiving laboratory specifications and shipped under appropriate conditions (containers, temperature, light) within 24 hours.

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Various commercially available sampling devices can be used to collect water samples at discrete depths or integrated samples collecting water from many depths. For example, water samplers are commercially available from Aquatic Ecosystems Inc. (www.aquaticeco.com) and Wildco (www.wildco.com). A Labline sampler can be used to take integrated water samples by lowering and raising the device at a constant rate between the depths of interest. The Labline sampler also allows discrete depth sampling, for example when physical parameters indicate fluorescence activity, elevated DO, or pH at a specific depth. A phytoplankton net tow is useful for collecting appreciable algal biomass from horizontal or vertical tows for qualitative determination of cell-bound toxin content. Net efficiency (depending on the mesh size and other features) needs to be considered in efforts to use these samples to estimate toxin content (Wetzel and Likens 2000). Water for toxin analysis should be collected in glass rather than plastic bottles to minimize toxin adsorption to the container walls. Toxin samples should be stored on ice during transport and filtered the same day of collection. The processed filtrate sample (soluble fraction) and the filter disc (cell-bound fraction) should be frozen separately at a minimum of –20°C to a maximum of –80°C (preferable; see appropriate references for further details, such as Chorus and Bartram 1999, Codd et al. 1999, and Meriluoto and Codd 2005).

FUTURE DIRECTIONS

The demand for drinking water will surely increase in the future due to population growth and the resulting development of municipal infrastructure. Watersheds will continue to be affected by a variety of anthropogenic and natural stressors, such as stormwater runoff due to increases in impervious surfaces, increases in harmful algal blooms, and extremes in climate. These synergistic stressors will increasingly challenge potable water treatment operations. The exceptional 2007 drought experienced by the southeastern United States (US Drought Monitor, www.drought.unl.edu/dm/monitor.html) provides a compelling example of how climate can quickly change the availability of water for the general public. The use of online monitoring and dedicated field techniques to assess source water for harmful algae will be a necessity to optimize management of decreasing water resources. Development of new online and field technologies is anticipated to grow in importance as a high priority for both government and private concerns.

The integration of molecular and analytical techniques with electronic sensors has great potential for future development and use in online monitoring programs. This emerging technology is based on analytical methods that detect specific toxic substances. Examples of these technologies include the electric cell-substrate impedance sensing system, which measures toxicant-induced changes in the electrical impedance of a cell monolayer (Keese et al. 1998), and the neuronal microelectrode array device, which evaluates changes in the action potential of a neuronal network via noninvasive extracellular recordings (Pancrazio et al. 2003). These sensors have been successfully employed in laboratory studies (van der Schalie et al. 2006), and future efforts are expected to include incorporation of this type of technology into online equipment and field deployment tests in source waters used for potable supplies.

It is also probable that additional municipalities will incorporate online monitoring into their water assessment programs in the future to assist managers and water treatment plant operators in planning for episodic events that negatively affect source water. The powerful information that can be gained from a robust online and field monitoring program will allow more accurate projections of costs related to water plant operations, such as the purchase of chemicals, filter material, and associated labor

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expenses. It is likely, as well, that manufacturing cost efficiency for online monitoring equipment will be realized by vendors. The resulting reduced initial monetary investment for the tools and associated software needed to implement this technology will make online monitoring an attractive addition to source water assessment programs for tracking harmful and noxious algal blooms that are expected to flourish in increasingly nutrient-enriched watersheds.

REFERENCES

American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF). 2005. *Standard methods for the examination of water and wastewater.* 21st ed. Washington, D.C.: APHA.

Arnell, N.W. 1999. Climate change and global water resources. *Global Environ. Change,* 9:S31–S49.

Botana, L.M., ed. 2007. *Phycotoxins: Chemistry and biochemistry.* Ames, Iowa: Blackwell.

Burkholder, J.M. 1992. Phytoplankton and episodic suspended sediment loading: Phosphate partitioning and mechanisms for survival. *Limnol. Oceanogr.,* 37:974–988.

Burkholder, J.M., W. Frazier, and M.B. Rothenberger. 2010. Source water assessment and control/treatment strategies for harmful and noxious algae. In *Manual M57, Algae: Source to treatment.* Denver, Colo.: AWWA.

Chorus, I., and J. Bartram, eds. 1999. *Toxic cyanobacteria in water: A guide to their public health consequences, monitoring, and management.* London: E&FN Spon.

Codd, G.A., I. Chorus, and M. Burch. 1999. Design of monitoring programmes. In *Toxic cyanobacteria in water: A guide to their public health consequences, monitoring, and management.* I. Chorus and J. Bartram, eds. London: E&FN Spon.

Falkowski, P.G., and J. La Roche. 1991. Acclimation to spectral irradiance in algae. *Jour. Phycol.,* 27:8–14.

Geider, R.J., and B.A. Osborne. 1987. Light absorption by a marine diatom: Experimental observations and theoretical calculations of the package effect in a small *Thalassiosira* species. *Mar. Biol.,* 96:299–308.

Glasgow, H.B., Jr., J.M. Burkholder, R.E. Reed, A.J. Lewitus, and J.E. Kleinman. 2004. Real-time remote monitoring of water quality: A review of current applications, and advancements in sensor, telemetry, and computing technologies. *Jour. Exper. Mar. Biol. Ecol.,* 300:409–448.

Grayman, W.M., R.A. Deininger, and R.M. Males. 2001. *Design and early warning and predictive source water monitoring systems.* Denver, Colo.: AWWA.

Keese, C.R., N. Karra, B. Dillon, A.M. Goldberg, and I. Giaever. 1998. Cell-substratum interactions as a predictor of cytotoxicity. *In Vitro Molec. Toxicol.,* 11:182–193.

Kirk, J.T.O. 1994. *Light and photosynthesis in aquatic ecosystems.* 2nd ed. Cambridge, UK: Cambridge University Press.

Knappe, D.R.U., R.C. Belk, D.S. Briley, S.R. Gandy, N. Rastogi, A.H. Rike, H. Glasgow Jr., E. Hannon, W.D. Frazier, P. Kohl., and S. Pugsley. 2004. *Algae detection and removal strategies for drinking water treatment plants.* Denver, Colo.: AWWA.

Lettenmaier, D.P., A.W. Wood, R.N. Palmer, E.F. Wood, and E.Z. Stakhiv. 2004. Water resources implications of global warming: A US regional perspective. *Clim. Change,* 43:537–579.

Lund, J.W.G., C. Kipling, and E.D. Le Cren. 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. *Hydrobiologia,* 11:143–170.

Meriluoto, J., and G.A. Codd, eds. 2005. TOXIC: Cyanobacterial monitoring and cyanotoxin analysis. *Acta academiae aboensis, Ser. B, Mathematica et physica,* Vol. 65, No. 1. Turku, Finland: Åbo Akademi University Press.

Mikol, Y.B., W.R. Richardson, W.H. van der Schalie, T.R. Shedd, and M.W. Widder. 2007. An online real-time biomonitor for contaminant surveillance in water supplies. *Jour. AWWA,* 99:107–115.

Milley, P.C.D., K.A. Dunne, and A.V. Vecchia. 2005. Global pattern of trends in streamflow and water availability in a changing climate. *Nature,* 438:347–350.

Pancrazio, J., S.A. Gray, Y.S. Shubin, N. Kulagina, D.S. Currino, K.M. Shaffer, K. Eisemann, A. Curran, B. Zim, G.W. Gross, and T.J. O'Shaughnessy. 2003. A portable microelectrode array recording system incorporating cultured neuronal networks for neurotoxin detection. *Biosens. Bioelectron.,* 18:1338–1347.

Schlüter, L., T.L. Lauridsen, G. Krogh, and T. Jørgensen. 2006. Identification and quantification of phytoplankton groups

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in lakes using new pigment ratios: A comparison between pigment analysis by HPLC and microscopy. *Freshwater Biol.*, 51:1474–1485.

Shedd, T.R., W.H. van der Schalie, M.W. Widder, D.T. Burton, and E.P. Burrows. 2001. Long-term operation of an automated fish biomonitoring system for continuous effluent acute toxicity surveillance. *Bull. Environ. Contam. Toxicol.*, 66:392–399.

Tillmanns, A.R., F.R. Pick, and R. Aranda-Rodriquez. 2007. Sampling and analysis of microcystins: Implications for the development of standardized methods. *Environ. Toxicol.*, 22:132–143.

Touchette, B.W., J.M. Burkholder, E.H. Allen, J.L. Alexander, C.A. Kinder, C. Brownie, J. James, and C.H. Britton. 2007. Eutrophication and cyanobacteria blooms in run-of-river impoundments in North Carolina, USA. *Lake Reservoir Manage.*, 23:179–192.

US Environmental Protection Agency. 1997. *Method 445.0. In vitro determination of chlorophyll a and pheophytin a in marine and freshwater algae by fluorescence.* Cincinnati, Ohio: USEPA.

van der Schalie, W.H., T.R. Shedd, P.L. Knechtges, and M.W. Widder. 2001a. Using higher organisms in biological early warning systems for real-time toxicity detection. *Biosens. Bioelecton.*, 16:457–465.

van der Schalie, W.H., T. Shedd, M. Widder, A. Kane, E. Silbergeld, R. Reimschuessel, M. Poli, C. Sarabun, J. Burkholder, and H. Glasgow Jr. 2001b. *Real-time monitoring for toxicity caused by harmful algal blooms and other water quality perturbations.* Technical Report EPA/600/R-01/103. Washington, D.C.: USEPA.

van der Schalie, W.H., R.R. James, and T.P. Gargan II. 2006. Selection of a battery of rapid toxicity sensors for drinking water evaluation. *Biosens. Bioelectron.*, 22:18–27.

Wetzel, R.G. 2001. *Limnology.* 3rd ed. New York: Academic Press.

Wetzel, R.G., and G.E. Likens. 2000. *Limnological analyses.* 3rd ed. New York: Springer-Verlag.

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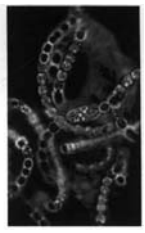

Chapter 2

Sampling and Identification: Methods and Strategies

Linda Ehrlich

There are numerous reasons for water utility managers, analysts, and operators to be interested in using biological methods to assess water quality. Many of the compliance and process issues facing utilities result from the growth of the aquatic primary producer organisms broadly termed "algae." These issues include total organic carbon (TOC) removal, filtration and coagulation, tastes and odors, and toxins. Algae may drift freely as phytoplankton in the water column of reservoirs, tributary streams, impoundments, and basins; or they may be attached to bottom sediments and other surfaces in those water bodies, in which case they are known as periphyton. The physical and chemical characteristics of water bodies affect the abundance, species composition, stability, productivity, and physiological condition of algal populations.

Conducting assessments of algal populations can provide information on a number of source water quality and treatment issues (see Section 10010, Biological Examination, *Standard Methods*, APHA et al. 2005). Compliance and daily operational issues constitute the primary category of treatment concerns for utilities. Operational issues include

1. Detecting early warning of biogenic turbidity and taste and odor episodes;
2. Monitoring for clogging of pipes, screens, and filters;
3. Measuring fluctuations in chemical parameters, such as alkalinity, pH, and oxygen; and
4. Determining the algal removal efficiency and effectiveness of drinking water treatment stages in order to optimize chemical dosages and physical treatment processes.

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Compliance issues include

1. Assessing the need for removal of disinfectant/disinfection by-product (D/DBP) precursors (i.e., TOC); and
2. Monitoring the presence and extent of algal toxins and the algae that produce them.

Longer-term issues, such as the following, constitute a secondary level of concern:

1. Evaluating the algal production potential of a source water to determine nutrient sensitivity.
2. Tracking long-term changes in biomass and species diversity, including "nuisance species," in order to assess the effects of human activities such as domestic wastewater discharge or runoff of fertilizers or harmful chemicals.
3. Documenting the short- and long-term variability in algal productivity caused by natural phenomena (e.g., drought, flooding, and climatic changes).
4. Identification of the extent and location of nutrient loading and pollutant inputs from the watershed.
5. Determining optimum times for treatment with algicides and monitoring treatment effectiveness.

This chapter deals with the process of properly designing algae monitoring and analytical protocols to address specific algae-related problems that can interfere with operations and compliance. It is intended to provide practical guidance to analysts, lab technicians, and biologists with little or no training in algae sampling, counting methods, and especially taxonomy. First, it is important to define the types of aquatic environments and algal communities that must be monitored.

AQUATIC ENVIRONMENTS AND ALGAL COMMUNITIES IN SURFACE WATER UTILITIES

A water utility may encompass multiple types of aquatic environments, from the initial surface water supply, which may be a river or reservoir, to intermediate impoundments and treatment basins within the plant. The hydraulic behavior of a basin can be classified as either lotic or lentic in nature and can be controlled to some extent if the flow in and out of the basin can be controlled. In studying algal communities, it is important to understand that variations in depth and water flow rate (residence time) create different microenvironments that can encourage some types of algal growth and suppress others. In fact, these environments, containing water originating from the same source, may support entirely different types and densities of algae. So, for a complete algal monitoring program, it is necessary to collect data from all types of aquatic environments in the utility.

Lotic environments are defined as flowing freshwater systems where there is unidirectional (advective) water movement along a slope in response to gravity, that is, streams or rivers (Wetzel 2001). Many water utilities draw their raw water directly from lotic sources. The continual flow in lotic waters limits most algal growth to periphytic forms attached to rocks, debris, and sediments. True riverine phytoplankton (potamoplankton) do occur, but their abundance is generally low in such environments (Hötzel and Croome 1999). Often, the phytoplankton in lotic waters is incidental, consisting mostly of periphyton dislodged from natural substrates or plankton released from an upstream impoundment. The various basins within a water treatment plant

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(WTP), while appearing to be standing ponds, sometimes behave more like rivers. Detention times vary from minutes to hours to even longer (L. Forkner, personal communication). Shorter detention times make the basins more lotic and thus more favorable environments for attached filamentous, stringy algae such as *Cladophora, Ulothrix, Stigonema*, and various diatoms. These may produce taste and odor, clogging, or in the case of some blue-green algae (cyanobacteria), toxin problems. In fact, in lotic environments, the most important water quality indicators are often the attached organisms.

Lentic environments are defined as calm, standing water basins. Continuous flow occurs within these basins, but at a much slower rate, resulting in relatively longer residence times. Thus, these systems are more subject to the seasonal establishment of stratified microenvironments due to reduced circulation. Water supply reservoirs are impoundments created by damming river valleys. Behind the dam, riverine flow is restricted; thus, the reservoir gradually takes on the characteristics of a lentic environment. In these relatively calmer waters, planktonic algae predominate and have adequate residence time to produce potentially excessive populations. Algal growth in the euphotic zone, or epilimnion, may progress until nutrients are reduced to growth-limiting concentrations. During stratification, algal cells may sink below the thermocline (metalimnion), the zone of steep density gradient separating stratified water layers. Most utilities withdraw water from their initial water supply (river or reservoir) into intermediate impoundments that may, if detention times are long enough (days), behave as lentic environments. Phytoplankton organisms in lentic environments have great value as water quality indicators (APHA et al. 2005).

There are three general types of primary producer communities associated with surface water bodies and water utilities: phytoplankton, periphyton, and macrophyton. For the purposes of this chapter, sampling methods are discussed only for the phytoplankton and periphyton.

Phytoplankton

Phytoplankton are considered to be aquatic algae that are nonmotile or weakly motile, essentially drifting with water currents or suspended in water. They thrive in relatively quiescent water bodies (i.e., ponds, basins, lakes, and reservoirs). They are generally small or microscopic in size. They belong to all of the taxonomic algal groups, including the cyanobacteria, or blue-green algae. They comprise the bulk of algae entering a treatment plant. Plankton may accumulate on treatment plant filter beds, causing clogging and breakthrough. Cyanobacteria, in particular, are important to water managers because of their potential toxicity to animals and humans, as well as aesthetic and ecological issues caused by their blooms (e.g., surface scums, low dissolved oxygen, adverse tastes and odors, and D/DBP precursors).

Periphyton

Periphyton are characterized as aquatic or terrestrial algae growing on surfaces of submersed objects (e.g., reservoir walls, filter beds, intake gates, rocks, soil, sediments, and aquatic macrophytes). They tend to be forms that colonize substrates in flowing water environments; thus, they constitute the dominant algal community naturally occurring in streams and rivers. However, they may detach and become part of the plankton. Periphytic algae are not limited to natural lotic environments, though. They may colonize numerous microenvironments within reservoirs and basins. They can grow on sediments and in shallow littoral zones and can clog intakes.

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SAMPLING

The importance of proper sampling cannot be overemphasized. Research has shown that random sampling error is the greatest source of error in estimating algal abundance (Lund et al. 1958). The process of obtaining useful qualitative and quantitative information about algal assemblages begins with four steps:

1. Thoughtful planning of sampling objectives (see list at top of chapter).
2. Knowledge of scientifically accepted sampling methods.
3. Use of appropriate water sampling equipment and sample containers.
4. Proper sample preservation.

At first, creating a sampling plan might appear to be a daunting task, given the variety of sample collection methods and the seemingly endless array of sampling equipment available. But the problem actually can be condensed to a matter of matching

1. The proper sampling strategy,
2. The proper sampling apparatus, and
3. The specific aquatic environment and algal community to be examined.

Sampling Strategies for Different Aquatic Environments

Because the character of surface water supplies is variable, as are treatment plant designs, sampling strategies must be individualized for each particular system.

Rivers. A utility whose water supply is a river most often collects surface grab samples for phytoplankton analysis at or near the point of water withdrawal. Sites can also be chosen upstream and downstream of a point source or ecological impact, such as a wastewater treatment plant, weir pool, or tributary. Ideally, the river should be sampled in the main channel to obtain data not overly influenced by local microhabitats, such as sloughs, inlets, or backwaters. The main channel is best accessed by boat or from a bridge. Sampling in the main channel may not be possible for large rivers, in which grab sampling from the riverbank by means of a dipper with a long extension (Wildlife Supply Company® 2007) will be necessary. A subsurface fixed-depth sample collected at 0.5 to 1 m below the surface is considered to be generally representative if river water is well mixed vertically. It is possible, though, to encounter horizontal or vertical thermal stratification due to thermal discharges or mixing of warmer or colder water from tributaries or reservoirs (APHA et al. 2005). Also, there may be "retentive zones," for example, pockets of riverbed where water is entrained and is incompletely mixed with the bulk of the flow (Hötzel and Croome 1999). Under conditions of incomplete mixing, collection of a depth-integrated sample from the main channel by boat is recommended, either by means of a pump with tubing or an integrating sampler. Temperature and dissolved oxygen profiles should be taken to verify stratification. It may be appropriate to collect several samples across the river to account for heterogeneity. Alternatively, samples can be collected from several slightly different spots at one site (Hötzel and Croome 1999; Vollenweider 1969). Samples should be taken as frequently as is feasible in order to detect population changes due to both seasonal variability and the influences of upstream events (e.g., flooding or releases from upstream impoundments). Plankton in rivers can originate from scouring of periphyton from natural substrates or from adjacent lakes, backwaters, and upstream reservoirs. The methods of sampling from different locations within a river are summarized in Table 2-1. Sampling for periphytic algae growing on river substrates occurs separately from phytoplankton sampling (see Periphyton Sampling Methods and Apparatus). It may be

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Table 2-1 Guide for matching sampling apparatus and sites in a river

	Location Within River		
Equipment	Shore	Bridge	Boat
Dip Stick (DS)	DS		
Discrete Depth Sampler (DD)		DD	DD
Integrated Sampler (IS)		IS	IS
Pump with tubing (P)			P

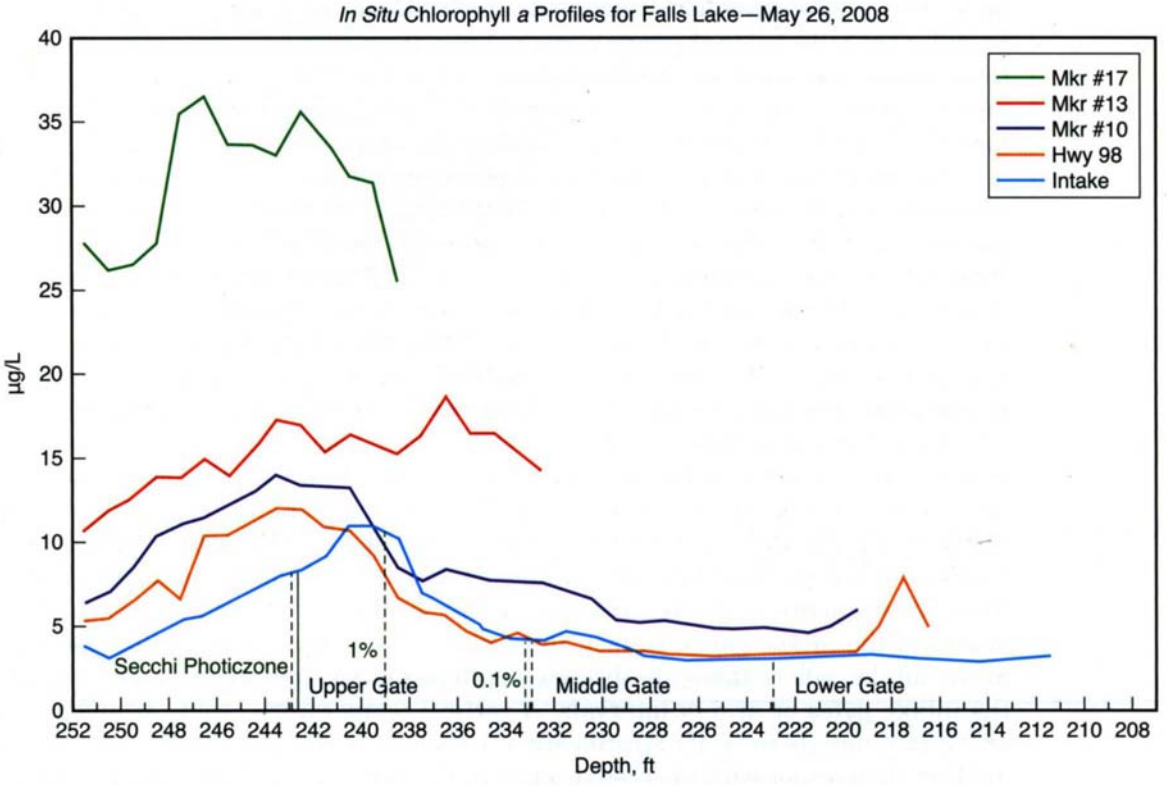

Figure 2-1 Horizontal and vertical gradients in *in vivo* chlorophyll *a* (Courtesy of Spirogyra Diversified Environmental Services, with permission of the City of Raleigh, N.C.)

advisable to sample river sediments and other natural substrates that may support periphyton growth.

Reservoirs. A reservoir shares riverine (lotic) and lacustrine (lentic) characteristics. A reservoir is designed to reduce flow rates, increase water residence times, and facilitate the settling of contaminants. Thus, significant horizontal and vertical gradients are produced with natural flow-related and seasonal variations (Figure 2-1). Moreover, flood control reservoirs are subject to abrupt adjustments in discharge below the dam that can introduce wide fluctuations in water level and further complicate internal circulation patterns. Thus, reservoirs often require more complex sampling strategies.

For monitoring horizontal gradients in a long, narrow reservoir, it is recommended that phytoplankton sampling be conducted at several points along a minimum of three regularly spaced parallel transects that are perpendicular to the long axis of

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the basin, with the first near the inlet and the last near the outlet (APHA et al. 2005). However, in a very large reservoir, and where resources are limited, it may be necessary to choose an alternative strategy, such as sampling at strategic points within the open water of the river channel along the longitudinal axis. Such points would include areas near intakes and discharges, inflows to the reservoir, constrictions within the reservoir, and major tributary arms that may influence the basin below confluence. The number of sampling sites and samples collected is determined by the study objectives, basin morphology, and financial resources. At a minimum, three sites should be chosen, including one near the inlet and one near the outlet. If there are major tributary arms, it is advisable to establish a site within each. Hötzel and Croome (1999) have provided drawings with suggested sample placement in reservoirs and weir pools. Samples are collected in the open water in order to avoid littoral zone populations, benthic species, and wind-accumulated scums. Also, bays and inlets that may be poorly mixed and have little water exchange with the main basin should be considered. They may develop a locally different water quality than that of the main basin.

In lentic environments, plankton populations can also vary vertically with depth, especially during periods of thermal stratification. In some instances, phytoplankton species such as the cyanophytes *Spirulina/Arthrospira*, *Cylindrospermopsis*, and *Planktothrix* may predominate in lower strata and never appear in the epilimnion (Figure 2-2). Ideally, phytoplankton samples should be collected from all major depth zones or water masses (APHA et al. 2005). If the water body is shallow, less than 2 m in depth, a single subsurface grab sample at 0.5 m may be sufficient. This would not be adequate in waters deeper than 2 m because the sample would not be representative of the vertical distribution of the phytoplankton population. Deeper waters would thus require a depth-integrated sample taken from a boat over the photic zone and/or several discrete depth samples (see Table 2-2). The number and depth of such samples will be determined by several factors: water depth at the site; depth of the photic zone (as determined by Secchi depth or radiometer [see Plankton Sampling Methods and Apparatus]); depth of the thermocline or chemocline; or, at a multilevel intake, the depths of the intake gates. In fact, there should be at least one location, most likely at the intake, where there are discrete depth sampling sites, at the various depths of the intake gates or at 5-m intervals. A vertical temperature profile should be taken at 0.3-m intervals to verify stratification. However, if it is not possible to collect and analyze samples for each microenvironment, the vertical variability and patchiness of phytoplankton populations can be integrated by collecting a composite sample. This can be done over the entire water column or, more commonly, over the photic zone. The photic zone is considered to be the uppermost stratum of the water column, containing 99% of the available light incident on the water surface. The depth of the water column at which 1% of the incident light remains is commonly assumed to equal twice the Secchi depth.*

There are several acceptable ways to collect a depth-integrated sample (see Plankton Sampling Methods and Apparatus). One simple way is to lower an integrating sampler through the water column of interest. Alternatively, a pumping system can be used where an attached length of flexible tubing fitted with a stainless-steel tubing weight is lowered through the depth of interest while water is pumped to a compositing container. The tubing can be lowered and raised continuously or paused at discrete depths to create a composite sample. If such samplers are not available, a number of grab samples can be collected with a discrete depth sampler, which are then pooled and mixed into an incremental composite. In eutrophic (nutrient-rich) waters,

* Hötzel and Croome (1999) cite 2.5× the Secchi depth from Schwoerbel (1970).

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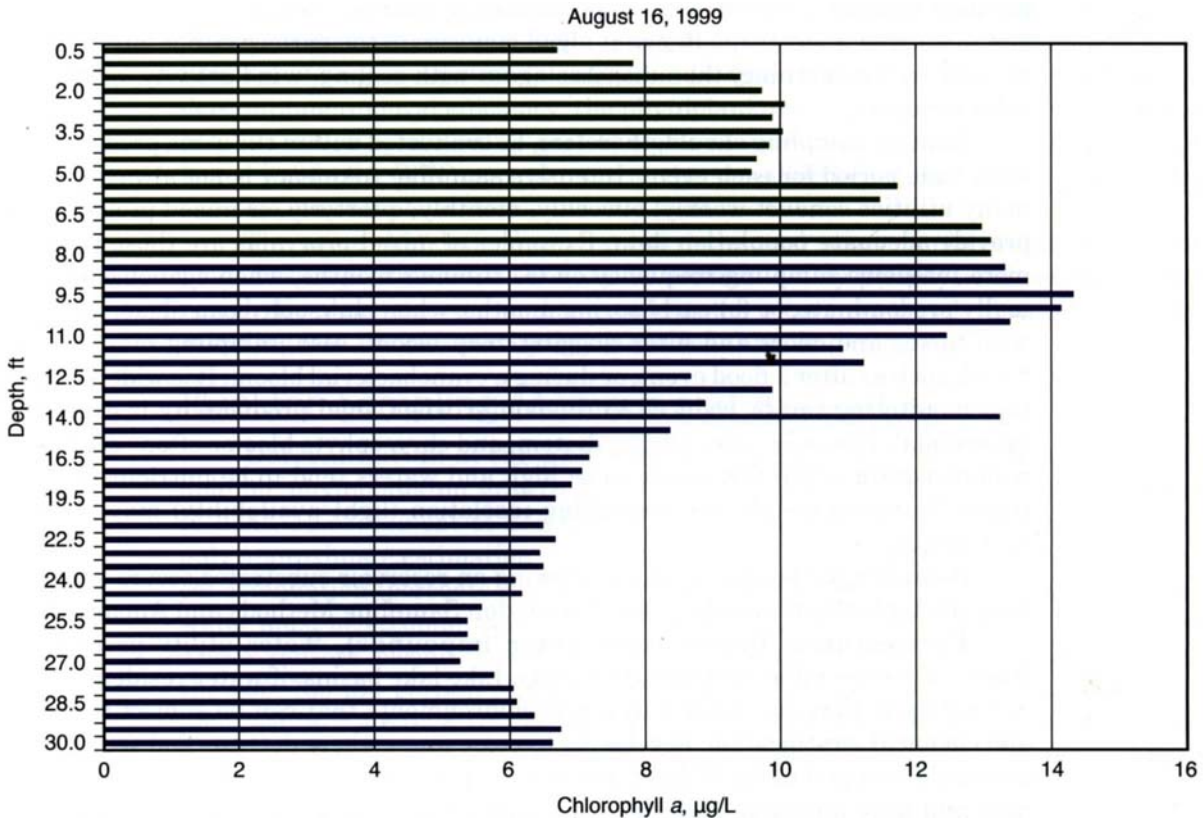

Figure 2-2 Vertical profile of *in situ* chlorophyll *a* at the intake, showing metalimnetic concentration of phytoplankton (Courtesy of Spirogyra Diversified Environmental Services, Burlington, N.C.)

Table 2-2 Use of sampling equipment in lentic environments

	Lentic Environment			
	Reservoir		Raw Water	
Equipment	Shallow	Deep	Impound	Plant Basin
Dip Stick (DS)			DS	DS
Discrete Depth Sampler (DD)	DD	DD	DD	DD
Integrated Sampler (IS)		IS	IS	IS
Pump with tubing (P)		P		

a sample size of 0.5–1 L should be adequate. However, in oligotrophic (nutrient-poor) waters, it may be necessary to collect as much as 6 L. Phytoplankton samples are always collected as whole water samples, unfiltered and unstrained.

The phytoplankton community is continually dynamic, so frequent sampling is desirable. Many taxa possess the mechanisms to migrate vertically in response to daily solar cycles. Often, species such as the cyanophyte *Cylindrospermopsis* will concentrate at the thermocline or chemocline. But they are also constantly subject to random horizontal migrations due to winds and currents. Thus, daily sample collection, and even collection at different times during the day and at different depths, provides the

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greatest amount of information on population dynamics. Phytoplankton are often subject to diurnal migrations. Buoyant algae may rise to the surface either late in the day or early in the morning, then disperse again with cooling, wind activity, or changing solar intensity.

Routine sampling should, therefore, be conducted within the same predetermined daily time period for each event. Intensive sampling frequency is not always possible; many utilities conduct weekly, biweekly, monthly, quarterly, or mixed programs that provide adequate population data. Examples of mixed programs are those that focus more intensive sampling frequency on (a) summer months, when cyanobacteria normally predominate, or (b) early spring months, when chrysophycean algae, associated with tastes and odors and filter clogging, may bloom. Also, sampling may be event-based, such as after a flood event, or during a cyanobacterial bloom. It is widely believed that monitoring can be reduced in the winter when algal productivity is expected to be minimal. However, cyanophyte, diatom, and chrysophyte blooms often occur during winter months when flow tends to be high and waters tend to be nutrient rich. Late winter warming trends and increasing insolation (light availability) may stimulate such blooms.

Sampling for periphytic algae growing on reservoir substrates occurs separately from phytoplankton sampling (see Periphyton Sampling Methods and Apparatus).

Pretreatment basins (raw water impounds). Water utility pretreatment basins often resemble circular lake basins. Like lake basins, if water residence time is several days, they can behave as lentic environments that can be subject to thermal and chemical stratification, developing hypoxic zones where detritus and sediment can accumulate appreciably. If detention time is long enough, planktonic algae predominate and have adequate residence time to produce potentially excessive populations. Generation times of algae vary from less than a day to several days (Hötzel and Croome 1999). Phytoplankton densities can change even more rapidly in a pretreatment basin than in a reservoir because of its relatively smaller size and mean depth, and variable retention times. It is not at all unusual for influent water with only moderate algal abundance to produce a sudden phytoplankton bloom and taste and odor episode while being held in a raw water impoundment. Also, when a river water plant is designed for sediment removal with two basins in series, less turbid growth conditions in the final basin can favor abrupt increases in phytoplankton productivity. It is recommended that circular basins be sampled at several points along two perpendicular transects extending from edge to edge (APHA et al. 2005). However, a more practical minimal design would involve three points along a transect extending from the basin inlet to the outlet, including the deepest point in the basin. Several types of samples may be required (i.e., fixed-depth, integrated composite, and surface grab). Raw water impoundments can be subject to growth of surface scum–forming cyanobacteria, for example, *Anabaena* or *Microcystis*, which may be wind-concentrated at the downwind edge of a basin. In this case, surface grab sampling may be appropriate for identification and toxin testing purposes. But it should be remembered that the colonies in the surface scum are not representative of the water column where the scum population originates.

Periphyton communities can also proliferate in pretreatment basins. Sampling for periphytic algae growing on basin substrates occurs separately from phytoplankton sampling (see Periphyton Sampling Methods and Apparatus section).

Plant basins. Conventional WTPs are designed as a series of basins containing physical or chemical unit processes that progressively clarify and disinfect raw source water. Each basin may appear to be a lentic environment. However, there is a slow, continuous flow through the plant that tends to wash or flocculate out most phytoplankton. The stages of a conventional WTP generally include preoxidation (ozone or

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permanganate), coagulation/flocculation/powdered activated charcoal, sedimentation, filtration, and disinfection. Each successive process results in incrementally greater removal of biogenic matter (plankton and bacteria) from the raw water influent. For determining removal efficiency, the phytoplankton community remaining in each unit can be studied by collecting grab samples at each stage. Grab samples are often collected inside the WTP control room at the sampling bench. Integrated or fixed-depth samples may also be collected directly from the basins.

The somewhat lotic flow characteristics and many surfaces within treatment plant basins provide ideal habitats for colonization by periphytic algae. Sampling for periphytic algae growing on basin substrates occurs separately from phytoplankton sampling (see Periphyton Sampling Methods and Apparatus).

Plankton Sampling Methods and Apparatus

Determination of the photic zone. The depth of the zone where light is available for phytoplankton growth (photic zone) depends on the transparency of water to light. Measurement of Secchi disk transparency is a simple and inexpensive but only approximate estimation of transparency. A truly quantitative evaluation of vertical attenuation (percentage transparency) and the spectral characteristics of light in water must be accomplished *in situ* with underwater quantum sensors (Wetzel 2001). These sensors permit measurement of photosynthetically active radiation within the 400–700 nm portion of the spectrum directly in μeinsteins/m^2-sec (equivalent to μmol $sec^{-1}m^{-2} = 6.02 \times 10^{17}$ photons $s^{-1}m^{-2}$) (LI-COR Inc. 2005). Instruments for measuring surface and underwater irradiance are available commercially (e.g., LI-COR, Lincoln, Neb.).

Wetzel and Likens (1991) provide a detailed explanation of solar radiant energy and its measurement. In trying to quantitatively define the photic zone, the physical structure of the photoreception unit is important. Secchi transparency is essentially a result of the reflection of direct incident light from its flat surface. Thus, to ensure that the sensor is measuring only the penetration of direct incident light (i.e., an "apples to apples" measurement), flat quantum sensors should be used that respond largely to direct incident light, instead of spherical or hemispherical sensors that additionally respond to laterally scattered light (Wetzel and Likens 1991).

There is no single accepted protocol for photometrically determining the depth of the photic zone. One method is to use only an underwater sensor and compare underwater readings (I_Z) at discrete intervals z to the value measured immediately beneath the water surface on the unshaded side of the boat (Wetzel and Likens 1991). In this case, the value immediately below the surface represents the total direct underwater irradiance, I_0. However, even immediately below the surface, there is a significant loss of light incident on the water surface, due to wave-mediated reflection and scattering. Thus, an alternative method preferred by this author is to measure surface irradiance (I_0) concurrently with underwater measurements (I_Z) by means of a separate terrestrial quantum sensor placed on an unshaded platform at the water surface. This is especially helpful when light conditions change rapidly, such as under rapidly moving cloud cover (Wetzel and Likens 1991).

In correlating Secchi disk transparencies with photometrically determined percentage transparencies, the depth intervals z corresponding to readings (I_Z) of 10%, 1%, and 0.1% of I_0 are of greatest interest. The depth of Secchi transparency roughly correlates to 10% transmission (Wetzel and Likens 1991, Wetzel 2001). Since light attenuation in water is a logarithmic function, a depth equivalent to twice the Secchi transparency approximates 1% transmission (see Sampling Strategies for Different Aquatic Environments). The photometrically defined photic zone often includes the

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depth of 0.1% transmission and thus will not correspond exactly with the Secchi defined photic zone. Moreover, though Secchi transparencies correlate well with percentage transmission, variability occurs with seasonal changes, size of suspensoids, and sensitivities of underwater quantum radiometers (Bukata et al. 1988, Wetzel 2001).

As mentioned earlier, sampling methods and the type of sampling equipment chosen should conform to the objectives of the study (Wetzel and Likens 1991). In this section, the variety of available plankton sampling equipment and their use will be discussed. A phytoplankton sample intended for quantitation (total cell counts, biomass determination) should always be a whole water sample, unfiltered and unstrained. Regardless of the type of study, a standardized field sampling data sheet should be devised for recording all samples and environmental measurements taken at each sampling site. The sheet should contain spaces for recording information including name and location of study, site, GPS coordinates, weather conditions, date and time, names of sampling personnel, water depth, gauge height, type of sample and sample depth, and Secchi depth. The sheet should also contain space for vertical profiling parameters.

Plankton nets. Plankton nets, first introduced in 1846, were designed for qualitative sampling of phytoplankton and zooplankton (Wildlife Supply Company 2007; APHA et al. 2005) (Figure 2-3). They are still widely used, especially for instructive purposes, to entrap and readily concentrate organisms directly from their water environment. Mesh sizes vary from 10 to 1,000 μm, depending on the size classes of organisms to be retained (Wildlife Supply Company 2007). The term *net phytoplankton* refers to the size class of phytoplankton retained by a mesh size of 76–80 μm. However, the size classifications listed in Table 2-3 (Wetzel 2001) show a large range of sizes for planktonic organisms.

Three types of tows are possible: vertical, oblique, and horizontal. Both vertical and oblique tows produce a composite sample, whereas horizontal tows collect a sample at a discrete depth. Vertical tows are preferable for collecting an integrated water column sample, but oblique tows may be needed in shallow water (APHA et al. 2005). See Section 10200 B, *Standard Methods*, for illustrations of plankton net designs and a detailed discussion of their use. All of the types are available from Wildlife Supply Company, Buffalo, N.Y.

Advantages: The plankton net is the simplest means of concentrating organisms from waters of low to moderate productivity (oligotrophic to mesotrophic), where densities are expected to be low. The nets can be used to filter large, potentially infinite, volumes of water. Mesh sizes of 10, 20, 63, 80, and even 118 and 153 μm will capture many nanoplankton, microplankton, and mesoplankton organisms, especially large colonial forms. *Standard Methods* states that aperture sizes from 64 to 76 μm will capture most net phytoplankton and nanoplankton (APHA et al. 2005). A somewhat oversimplified guide for selection and seasonal use of freshwater net aperture is published in the Wildlife Supply Company Catalog (Table 2-4). A plankton net may be tossed in an overhand motion from a boat, platform, or shore so that the downward arc brings the mouth of the net into contact with the water surface first. Alternatively, it can be lowered into the water.

Nets are sometimes preferred where plankton populations are low or where only very limited qualitative (identification only) data are desired.

Disadvantages: Plankton nets are not recommended for thorough qualitative studies or any quantitative analyses of phytoplankton. As seen from Table 2-3, a large percentage of important nanoplankton and picoplankton can pass through the mesh openings of even the finest mesh size (APHA et al. 2005, Wetzel and Likens 1991). Moreover, the volume of water passing through most nets is very difficult to measure.

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Figure 2-3 Phytoplankton net (Courtesy of Wildlife Supply Company, with permission)

Table 2-3 Size classification of plankton

Term	Size Ranges (μm)
Femtoplankton	0.01–0.2
Ultraplankton	0.2–20
Picoplankton	0.2–2.0
Nanoplankton	2.0–20
Microplankton	20–200
Mesoplankton	200–200,000

Table 2-4 Selection and seasonal use of freshwater plankton nets

Organisms	Net Type	Season
Phytoplankton		
153 μm	Standard	Late summer, fall
80 μm	Fine	Late spring, early summer
Diatoms		
10 μm	Ultrafine	All seasons

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Water collection bottles. Thorough qualitative and all quantitative analyses require the collection of whole (unfiltered and unstrained) water samples (APHA et al. 2005). This can be accomplished with a water collection bottle consisting of a cylindrical tube with closures at each end and a triggering device. The open sampler is lowered carefully through the water column to a desired depth where it is then closed by dropping a weight, called a messenger. The messenger slides along a supporting cord and trips the closing mechanism. There are a number of sampler designs commonly used, but the Kemmerer and Van Dorn samplers are most often recommended for evaluations of phytoplankton (APHA et al. 2005).

The Kemmerer operates on a principle similar to the Van Dorn but has a different type of trip and operating mechanism. Kemmerer samplers are commonly manufactured of stainless steel but are also available in transparent acrylic, opaque PVC, or Teflon® for trace organic or trace metal sampling. There is also a long, thin style designed just for wells. Kemmerer samplers are designed for vertical sampling only (Figure 2-4).

Van Dorn bottles are designed to be used either for vertical or horizontal sampling, depending on the sampling objective. This is an important distinction, because the vertical and horizontal tubes have different flow characteristics that affect the accurate isolation of the community of interest. Vertical designs allow free flow of water through the cylinder during descent, so they do not significantly disturb water strata (Figure 2-5A). Thus, they are preferred for standing crop and primary productivity evaluations to accurately capture a sample at a fixed depth. The horizontal design is also intended for sampling at specific depths, but horizontal bottles descend horizontally, parallel to the bottom (Figure 2-5B). They are ideal for sampling narrow stratification layers, especially at the thermocline or just above the bottom sediments. To capture the appropriate sample with them, they are pulled sideways about 1 m before closing. A variation of the vertical Van Dorn sampler, the series sampling bottle, can be placed in a series of as many as five on a supporting line and closed simultaneously to sample as many as five discrete depths. Van Dorn samplers are also available in two styles, Alpha™ and Beta™. The differences are in the end seals and latex closing tubing; the Beta style is better suited to sampling trace metals and other chemicals (Wildlife Supply Company 2007).

Advantages: Water collection bottles can be lowered to a precisely determined depth to capture a repeatable, fixed sample volume. No power supply is required. They can be used from structures high above water surfaces (e.g., bridges or intake towers). The metal Kemmerer samplers are rugged and require little maintenance. Kemmerer samplers with Teflon bodies work well in severe environments. Acrylic bottles allow the contents to be viewed. Thermometers may be mounted on the bottles for instantaneous temperature measurement. A variety of sizes are available, from 0.4 L to 6.2 L. Both Kemmerer and Van Dorn samplers are well regarded by limnologists and have been used and recommended for scientific studies for decades (Sournia 1978).

Disadvantages: Both Van Dorn and Kemmerer samplers are relatively expensive. The specificity in materials and designs means that multiple types are likely to be required in order to be prepared for all possible sampling situations. Latex closing tubing and polyurethane seals eventually decay and must be replaced. Latex tubing and polyurethane seals leach some heavy metals. Samples may react with the sampler, causing errors. Metal and glass may dissolve into the sample, and plastics may leach metals from ultraviolet inhibitors and metalorganic plasticizers. Water collection bottles can be somewhat awkward to use. The trigger mechanisms are very sensitive and can trip prematurely. The support line must remain taut to allow the messenger to work properly; long airdrops may reduce the effectiveness of the messenger.

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Figure 2-4 Metal Kemmerer sampler (Courtesy of Wildlife Supply Company, with permission)

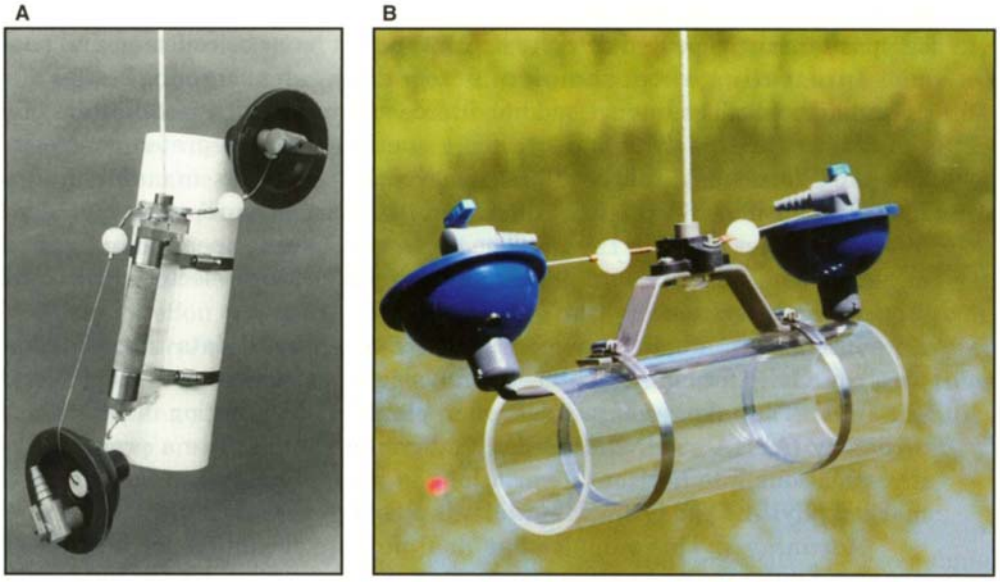

Figure 2-5 Van Dorn sampling bottles: (A) vertical, (B) horizontal (Courtesy of Wildlife Supply Company, with permission)

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Figure 2-6 Integrating water sampler (Courtesy of Thermo Fisher Scientific, with permission)

If composite samples are to be collected with these bottles, they must be incremental composites (i.e., repeated discrete depth samples must be collected and pooled).

Integrating water samplers. Integrating water sampling bottles are also vertical cylindrical tubes but are modified in design to permit the collection of water from the surface to a particular depth for a continuously integrated composite plankton sample (Wetzel and Likens 1991). One such sampler is manufactured by Thermo Fisher Scientific USA (Figure 2-6). It is a two-piece polypropylene cylinder with small inlet and outlet ports in the lid. The ports may be left open for integrated sampling or closed with accompanying plugs and opened at a preselected depth by means of a "spring-catch" system.

Integrated samples may provide more meaningful data than single-depth samples, especially for long-term trend analysis and statistical modeling (Wetzel and Likens 1991). They are recommended when plankton distribution is known to be variable or patchy (APHA et al. 2005). Also, integrated samples are a practical necessity for investigations into horizontal variations in populations, as in large reservoirs where marked physical and chemical gradients exist (Wetzel and Likens 1991).

Advantages: This sampler has dual-purpose flexibility for both integrated and discrete depth water sampling (Figure 2-7). Further, it can also be used for biochemical oxygen demand testing applications. Polypropylene construction allows accurate testing for trace metals. The built-in weighted base provides stability.

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Figure 2-7 Use of an integrating sampler off of a boat

Disadvantages: The integrating sampler is also relatively expensive and is limited to vertical sampling. There are only two sizes available: 1.4 L and 2 L.

Pumps. Another accepted sampling device for whole water sampling is the pump. Only diaphragm and peristaltic pumps are recommended, as they are less damaging to organisms than centrifugal pumps (APHA et al. 2005) (Figures 2-8 and 2-9). A weighted hose, attached to a suction pump, is lowered to the desired depth so that water can be pumped to the surface. For samples to be analyzed for organochlorine compounds, tetrafluoroethylene (TFE) tubing should be used. Pumps are essential for *in vivo* fluorometry applications, where water must be pumped from a selected depth through the cell of a field fluorometer for estimates of *in vivo* chlorophyll (Turner Designs Inc., Sunnyvale, Calif., USA).

Advantages: Pumps provide greater speed of collection and thus are advantageous when it is necessary to obtain large, accurately measured quantities of organisms. Continuous integrated composite samples can be collected. The pump also provides dual-use flexibility in that it can supply a homogeneous sample from a given depth or an integrated sample from the surface to a particular depth (APHA et al. 2005). Pumps are durable, low maintenance, and can be relatively light and compact.

Disadvantages: The pump is the most expensive of the sampling options discussed. An external power supply, usually a DC battery, is required. Peristaltic pumps utilize two types of tubing, usually flexible silicon pump head tubing connected to more rigid Teflon or Tygon® "down" tubing. Thus, appropriately sized tubing connectors are necessary, made of stainless steel or rigid plastic. Pump head tubing eventually needs to be changed. It may be prudent to change both pump head and down tubing between sampling sites to avoid cross contamination of samples. A tubing weight is also necessary to ensure proper descent of the tubing.

Periphyton Sampling Methods and Apparatus

Natural substrates. Periphyton, algae that grow attached to submerged living and nonliving surfaces, are notoriously difficult to collect from their natural substrates. The approved method (Porter et al. 1993, APHA et al. 2005) for removing qualitative

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Figure 2-8 Use of the Solinst® peristaltic pump (Courtesy of Solinst Canada Ltd., Georgetown, Ontario, Canada)

Figure 2-9 Use of the Teledyne Isco compositing sampler with peristaltic pump (Courtesy of Teledyne Isco, Inc.)

samples from rocks and other substrates is by scraping the substrate with a periphyton brush over a flat-bottomed macroinvertebrate pan (Wildlife Supply Company 2007). If a quantitative sample is desired, a measured standard surface area must be marked on the substrate and scraped. After removal, material is then washed into a vial or Whirl-Pak™ bag, preferably with water from the sample environment. Samples may be examined fresh or preserved. Samples may be examined initially, while still attached to the substrate, with a hand lens or stereomicroscope. Subsequently, definitive identification may require examination with a compound light microscope.

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Figure 2-10 Various Hester-Dendy periphyton samplers (Courtesy of Wildlife Supply Company, with permission)

Artificial substrates. An easier and more controlled means of obtaining a quantitative periphyton sample over a standard surface area is to use an artificial substrate. There are two types commercially available and approved by the US Environmental Protection Agency (USEPA): the Hester-Dendy multiplate substrate sampler and the Wildco® periphyton sampler (Wildlife Supply Company 2007; APHA et al. 2005).

The Hester-Dendy sampler consists of a series of round or square natural Masonite plates assembled onto a bolt by a wing nut (Figure 2-10). It is suspended or attached to a submerged object for at least two weeks to allow colonization to take place. Placement of five samplers is recommended to provide sufficient replication (Wildlife Supply Company 2007, APHA et al. 2005). A Teflon policeman (Wildlife Supply Company 2007) is used to scrape algae from the plates.

The Wildco periphyton sampler consists of a tray that holds standard glass slides for colonization (Figure 2-11). This is the most widely used type (APHA et al. 2005). The slides can be removed and examined directly for identification and counting without scraping. At least five slides should be exposed to account for variability caused by normal differences in colonization of individual slides (APHA et al. 2005).

Also, an artificial substrate basket is available that can contain natural substrate materials for colonization. However, no community on an artificial substrate is representative of the natural community (APHA et al. 2005). Length of substrate exposure and seasonal changes in environmental conditions may affect sample composition (APHA et al. 2005).

Sample Containers

Water samples for phytoplankton identification or enumeration (counting) should be transferred immediately from the sampling apparatus or composite container to an appropriate container for transfer to the analytical laboratory. It is advisable to split samples into two aliquots, one unpreserved for observation of live organisms and one preserved for longer-term storage or quantitation. These two types of aliquots require different sample container types. Unpreserved samples may be placed into clean,

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Figure 2-11 Wildco periphyton sampler (Courtesy of Wildlife Supply Company, with permission)

translucent polyethylene bottles. Preserved samples should be placed into dark brown glass, PET (polyethylene terephtalate), or opaque HDPE (high-density polyethylene) bottles because of the sensitivity of the preservative to degradation by light. Silica leaching into solution from glass containers may help preserve diatom walls (Martin 1999). Polypropylene bottles are unsuitable because fumes from the most commonly used preservative, Lugol's iodine, will diffuse through them (Hötzel and Croome 1999). The sample bottle should be rinsed thoroughly with excess sample water. Containers should be labeled immediately with a durable label containing the following information: study location, sample site, sample type (preserved photic zone composite, unpreserved photic zone composite, fixed-depth, net, etc.), depth, date and time, and name of sampler.

Preservation

As mentioned above, an aliquot of the sample should be retained in a separate container and held in the dark, chilled to 10°C for observation of live organisms. This is helpful in determining certain diagnostic characteristics, such as motility and natural color. Live samples should be examined within 12 hours after collection because water quality can change within hours, and zooplankton grazing can alter the algal population (Hötzel and Croome 1999). The remainder of the sample should be preserved for longer storage. The most suitable algal preservative is Lugol's iodine solution, added to samples to yield a 1% final solution (1:100 V/V) (Wetzel and Likens 1991, APHA et al. 2005, Hötzel and Croome 1999). This ratio gives the solution a weak tea color (see Figure 2-12). A few extra drops of Lugol's may be added to samples every few months

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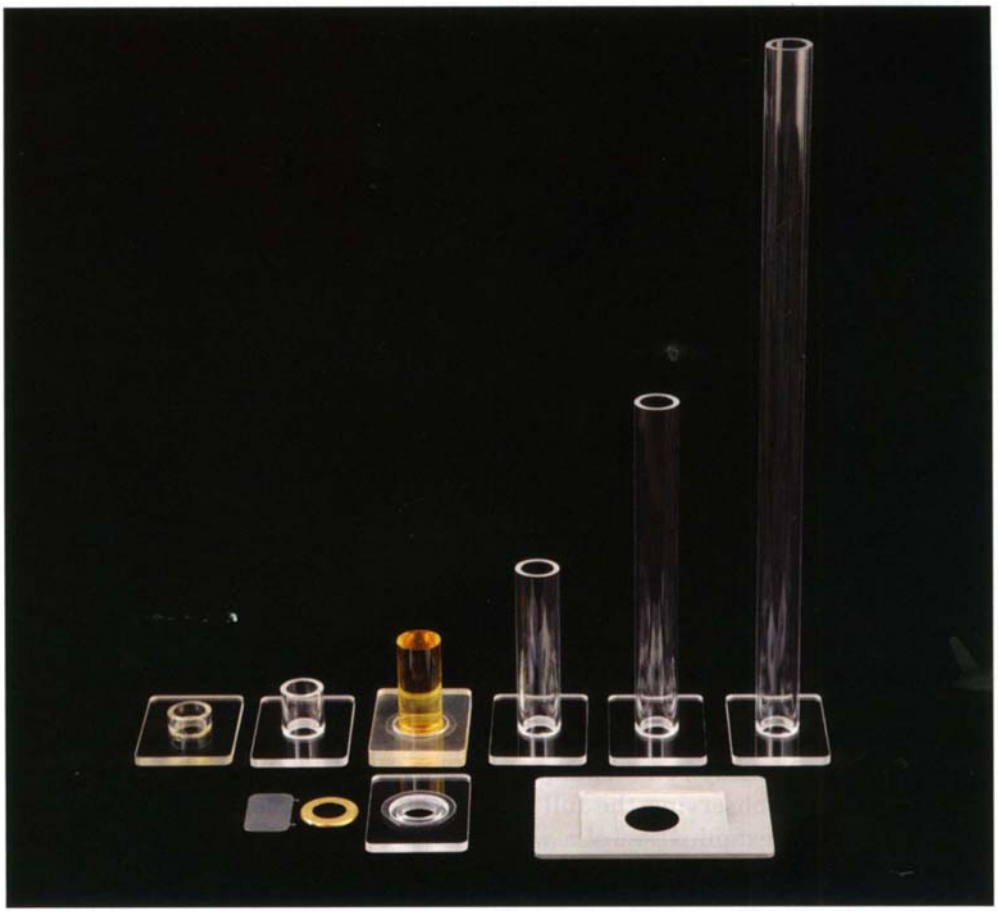

Figure 2-12 Utermöhl sedimentation chambers (Courtesy of Spirogyra Diversified Environmental Services, Burlington, N.C.)

for prolonged storage. Samples will keep for several years if stored correctly in the dark. Lugol's solution may be prepared by mixing 20 g of potassium iodide (KI) with 200 mL distilled water, then dissolving 10 g of pure iodine in this solution. Then glacial acetic acid (20 mL) may be added to enhance the longevity of preserved samples. A small amount of glycerol may also be added to Lugol's-preserved samples to prevent drying during long-term storage (Wetzel and Likens 1991). The stock solution should be stored in glass in a dark, well-ventilated space. A commercial preparation of Lugol's solution without glacial acetic acid is available from Carolina Biological Supply Company, Burlington, N.C.

There are a number of other preservatives described, for example, buffered formalin, glutaraldehyde, or merthiolate (Wetzel and Likens 1991, APHA et al. 2005, Hötzel and Croome 1999); however, they have serious disadvantages. Formaldehyde tends to throw off flagella and distort, rupture, or shrink delicate algae such as *Euglena, Cryptomonas, Chromulina, Synura,* and *Mallomonas* (APHA et al. 2005, Hötzel and Croome 1999, Wetzel and Likens 1991). Moreover, formalin and glutaraldehyde are highly toxic (carcinogenic) and must be used only with a fume hood. Lugol's solution is the preferred preservative for several reasons. Even though iodine is a toxicant, the major hazard of Lugol's solution is as an inhalation irritant (Carolina Biological Supply Co. 2000). It preserves flagella and is the least damaging to delicate forms. Further, the absorption of iodine from Lugol's preservative stains the cell and increases

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specific weight so that sedimentation is enhanced. In fact, phytoplankton samples to be concentrated and analyzed by the sedimentation technique should be preserved with Lugol's solution (Wetzel and Likens 1991).

If, however, material is to be sent to experts for further identification with an electron microscope, buffered glutaraldehyde fixative (2% GTA with sodium cacodylate of borate/borax or combined buffered glutaraldehyde/osmium tetraoxide cold fix [GTA/OsO_4]) or a weak formalin solution may be recommended (Martin 1999). The lab performing the electron microscope work should be consulted regarding the preferred preservation method.

ANALYTICAL METHODS

Unless the study objectives are extremely limited (e.g., tracking a cyanophyte bloom), most quantitative studies assess the entire phytoplankton community with, minimally, a total cell count. After collection, specimens are prepared for observation, identification, and enumeration (counting) with either a standard upright compound or inverted compound microscope.

Preparation Techniques for Phytoplankton Samples

In order to obtain a thorough examination, whether qualitative or quantitative, the organisms in a water sample usually must be concentrated prior to analysis (APHA et al. 2005, Wetzel and Likens 1991). Surface waters and treatment plant basins may perennially or seasonally be oligotrophic (minimally productive) environments. Thus, concentration may be necessary to produce statistically valid counts and increase the likelihood of observing the full species diversity of the assemblage being studied. A preliminary examination of a whole water sample often indicates the degree of concentration necessary. Some authors suggest that samples with a cell density less than 100 cells/mL require concentration (Hötzel and Croome 1999). There are three concentration techniques accepted by *Standard Methods:* sedimentation, membrane filtration, and centrifugation (APHA et al. 2005).

Sedimentation. Overall, the method preferred by most researchers is sedimentation because it allows organisms to settle by gravity and not be subjected to potentially disrupting vacuum or pressure forces; thus, it is nondestructive (Wetzel and Likens 1991, APHA et al. 2005, Hötzel and Croome 1999, Martin 1999). It is also considered to be nonselective, even though many of the picoplankton, smaller nanoplankton, and gas-trapping cyanobacteria may not settle completely (APHA et al. 2005, Wetzel and Likens 1991). Hötzel and Croome (1999) describe a method for collapsing the gas vacuoles of buoyant cyanophytes, but this type of manipulation may alter intracellular structures that have taxonomic value. A further advantage of sedimentation is that the algae settle in an essentially random distribution, which is necessary for statistically valid enumeration. The method was originally described by Utermöhl (1958) and later by Hasle (1978).

Preservation with Lugol's solution is important for samples concentrated by sedimentation, as the absorption of iodine from Lugol's fixative adds weight to the cells, thereby accelerating their sedimentation. Quantitative settling by gravity can be accomplished with a standard graduated measuring cylinder; however, there are chambers specially developed for this purpose. Sedimentation chambers, originally developed by Utermöhl (Lund et al. 1958), are available commercially from a number of suppliers (e.g., Hydrobios, Germany; Spirogyra Diversified Environmental Services, Burlington, N.C.). They are constructed from cast acrylic tubing (vertical tower) placed and temporarily sealed over a flat acrylic base unit that holds a circular, ultra-thin

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coverslip (Figure 2-12). Algae settle onto the glass coverslip and can be viewed on either a conventional or an inverted microscope.

Use of the Utermöhl chamber has several advantages over other settling methods. For example, after settling in a graduated cylinder, the supernatant liquid must be carefully siphoned off. There is a high likelihood of introducing subsampling error if cells are inadvertently lost during siphoning. More losses may occur during removal of the concentrated subsample. With the Utermöhl chamber, the tower, temporarily attached to the base, slides off of the base, leaving the cells undisturbed in the base unit. The concentrated subsample can be efficiently transferred to another type of counting chamber, or the base unit itself may be placed directly on the stage of an inverted microscope. Some of the shorter tower styles allow the entire assembly to be moved to the stage of an inverted microscope for examination without tower removal. This is made possible by the design of the inverted microscope, where objectives are placed below the microscope stage.

Utermöhl chambers are available in a range of nominal volumes: 5, 10, 15, 25, 50, and 100 mL. The selection of sedimentation chamber size is based on the suspected cell density of the sample. Samples from oligotrophic waters or finished drinking waters may require settling volumes of 50–100 mL, whereas more productive waters with greater phytoplankton densities may require only 5–10 mL. Any sized chamber that produces the desired level of precision is acceptable. However, the results obtained using different sized chambers will not be strictly comparable because of differences in physical settling characteristics associated with chamber height. In tall chambers, errors may be introduced with cells adhering to the walls or settling may be impeded by convection currents (Hötzel and Croome 1999). Thus, many authors, including this one, report that the overall number of species detected and the cell numbers may differ considerably between chambers.

After the base and cylinder are sealed with high vacuum grease into a single unit, the sample container is gently but thoroughly mixed by inversion for a minimum of 30 seconds. An aliquot of the sample is poured into a cylinder, filling the cylinder to leave a convex bead above the top edge. A second coverslip is slid across the top of the cylinder to remove the excess sample and enclose the exact sample volume with a vacuum seal. The chamber should be placed in a vibration free area during sedimentation. Settling times are based on chamber height and type of plankton. For most algae, chambers should sit undisturbed for at least 2 hours for each centimeter of water column at 20°C. The rule, as stated by Wetzel and Likens (1991), is that sedimentation time in hours should equal at least three times the height of the chamber in centimeters (see examples in Table 2-5). If small centric diatoms are known to be present, a settling time of 6 hours per centimeter of water column is recommended (Furet and Benson-Evans 1982).

After sedimentation, the sedimentation tower must be carefully slid off of the base plate to allow the supernatant to drain over a sink or some other collection vessel. As stated earlier, the base containing the concentrate can then be carefully moved directly to the stage of an upright or inverted microscope. Also, the shorter chamber assemblies (e.g., 5–25 mL) can be examined in their entirety on the stage of an inverted microscope. A potential disadvantage of examining a settled sample within the Utermöhl chamber can occur when samples contain high levels of sediment particles or floc (rivers, primary pretreatment basins, flocculation basins). Fine particles may be in the same size range as algal cells and can obscure the cells. Thus, it may be advantageous to mix the concentrate with gentle pipette suction and then transfer it to a different counting chamber.

Detailed discussions of the sedimentation technique are presented in *Standard Methods*, Wetzel and Likens (1991), Lund et al. (1958), Hötzel and Croome (1999), and Furet and Benson-Evans (1982).

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Table 2-5 Utermöhl chamber sizes and settling times (Courtesy of Spirogyra Diversified Environmental Services, Burlington, N.C.)

Chamber Size (mL)	Assembly Height (cm)	Settling Time (hr)	Settling Time (hr) (Utermöhl 1958)
5	2	4–6	
10	4	8–12	3
15	6	12–18	
25	11	22–33	
50	21	48–72	
100	42	96	18

Membrane filtration. Plankton organisms may be concentrated by filtration onto a membrane-type filter. A defined volume of sample is filtered through a cellulose membrane filter of 0.45-μm pore size at low vacuum pressure to a point where the filter is still just wet. The algae are then brushed off and resuspended in 5 mL of distilled water (Hötzel and Croome 1999). A 1-mL subsample is transferred to a Sedgewick–Rafter cell for observation. This method of concentration can damage fragile cells and can produce only a semiquantitative analysis (Hötzel and Croome 1999). However, it may be useful for quick operational needs, such as monitoring an algal bloom (Hötzel and Croome 1999).

The method of filtration onto a surface that can be rendered transparent permits the use of high magnification for identifying and enumerating picoplankters, flagellates, and nonsettling cyanobacteria (APHA et al. 2005, Wetzel and Likens 1991, Weber 1973). A mixed sample is poured into a funnel equipped with a membrane filter having a pore diameter of 0.45 μm. A vacuum of less than 50 kPa is applied to the filter until about 0.5 cm of sample remains on the filter. Then, low vacuum (~12 kPa) is applied to remove remaining water (APHA et al. 2005). The filter is then cleared and mounted permanently on a slide prior to examination with an upright compound microscope (St. Amand 1997).

Although this concentration method increases the probability of observing less abundant forms, it is not preferred for several reasons. Delicate forms such as "naked" flagellates are distorted by even gentle filtration. Filters are clogged quickly when samples contain dense plankton populations or high concentrations of detritus or silt. Silt particles may crush organisms or obscure them (APHA et al. 2005). Picoplankters smaller than 0.45 μm may be forced through the pores of the membrane, even with low vacuum. Enumeration of membrane filters is confounded by the nonrandom distribution of organisms on the filters (Wetzel and Likens 1991). The statistical validity of particle (unit) counts is predicated on the assumption of random distribution of natural units (Wardlaw 1985).

Centrifugation. Plankton can be concentrated quickly by batch or continuous centrifugation. To produce a concentrated pellet of most of the suspended particles, batch samples should be centrifuged at 1,000 g for 20 minutes. Although centrifugation accelerates sedimentation, it may damage fragile organisms (APHA et al. 2005). Its use is strongly discouraged for quantitative analyses because concentration of cells and colonies into even a loose pellet disrupts the random distribution of natural units on which statistical counting methods are based. However, centrifugation can still be a helpful tool when a quick survey is needed for a sample from oligotrophic (relatively unproductive) water. It is also a necessary part of the processing of a diatom sample prior to making a permanent mount.

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Dilution. Rarely, cell density in a sample may be high enough to require dilution instead of concentration in order to analyze the sample efficiently. In a very dense sample, smaller cells may be overlooked and counting may take too long. Counting errors increase when there are too many units in the field of view. In such a case, careful dilutions should be made and the dilution factor should be taken into account in the calculation of final results.

Preservation and Preparation of Periphyton Samples

Preservatives recommended for periphyton samples include 5% neutralized formalin, Lugol's iodine, or merthiolate (APHA et al. 2005). See the sections on Sample Containers and Preservation for a discussion of the various preservatives. Scrapings from natural substrates are preserved in liquid in wide-mouth containers made from the same materials as those for phytoplankton. Slides from artificial substrates can be preserved intact in such containers, or they can be scraped into the containers in the field (APHA et al. 2005). Periphyton should be removed from slides with a razor blade and rubber policeman. The scrapings should be transferred into at least 100 mL of liquid with preservative and disperse with vigorous shaking or with a blender (APHA et al. 2005, Acker et al. 2003a). The sample volume should be recorded.

Periphyton samples can be stained with either tetrazolium violet solution or acid fuchsin to permit distinguishing algae from detritus (see detailed discussion in *Standard Methods*, APHA et al. 2005).

Observation

Once a sample is concentrated, the next step in identification of algal organisms is to place all or a subsample of the concentrate into some type of slide mount for microscopic observation. An upright or inverted microscope equipped with 10×, 20×, 40×, and 100× achromatic objectives is required. Phase contrast objectives enhance the visualization of cellular structures. A Whipple reticule should be placed in one of the eyepieces in order to obtain cellular measurements and define an area for counting.

Wet slide mounts. A wet slide mount can be as simple as transferring an aliquot of concentrate with a pipette to a standard glass slide and covering it with a coverslip (APHA et al. 2005). The slide can be made semipermanent by ringing it with an adhesive such as clear nail polish. Temporary wet mounts can also be made with other specialized types of slides (e.g., Sedgewick–Rafter and Palmer–Maloney counting cells and the medical hemacytometer; APHA et al. 2005, Acker et al. 2003b, Weber 1973) (Figures 2-13, 2-14, and 2-15). All of these mounts can be placed on the stage of an upright compound microscope equipped with long working distance objectives for observation of morphological taxonomic features and some cellular structures. The Sedgewick–Rafter cell is also used with the inverted microscope. Cellular structure is best seen at high magnification (1,000×). Only the standard glass slide is suitable for very high magnification work. Wet slide mounts can be used for observation of both phytoplankton and periphyton.

Sedimentation chambers. Sedimentation chambers, used in combination with an inverted microscope, can eliminate the step of preparing a separate wet mount of the concentrated sample (Acker 2003, Weber 1973). They can be used for both phytoplankton and periphyton samples. Sedimentation chambers are manufactured with nominal volumes of 1, 5, 10, 15, 25, 50, and 100 mL (Figure 2-12). The four smallest chamber assemblies, 1, 5, 10, and 25 mL, can be transferred directly to the stage of an inverted compound microscope, a specialized microscope designed with the positions of the objective and condenser reversed in relation to the stage (Figure 2-16). The inverted design makes it possible to observe specimens through the bottom of containers such

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Figure 2-13 Gridded Sedgewick–Rafter counting cell (Courtesy of Wildlife Supply Company, with permission)

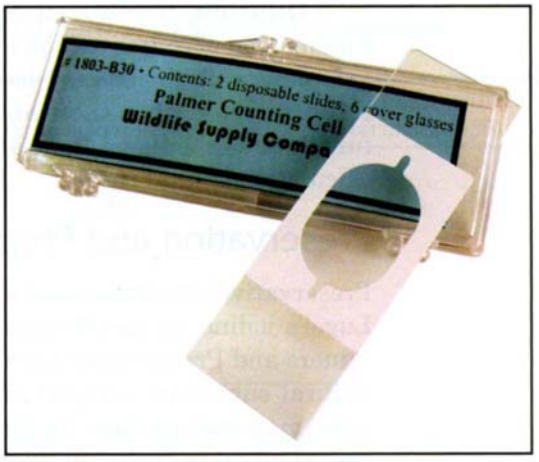

Figure 2-14 Palmer–Maloney counting cell (Courtesy of Wildlife Supply Company, with permission)

Figure 2-15 Medical hemacytometer (Courtesy of Wildlife Supply Company, with permission)

as sedimentation chambers. With these special chambers, fitted with ultrathin coverslips, the resolution of the inverted microscope can be extended to include organisms smaller than 10 μm (Wetzel and Likens 1991).

Phytoplankton permanent mounts. When the membrane filtration method of concentration is used, the filter must be rendered transparent and mounted permanently on a slide prior to examination. Filters can be cleared with either immersion oil or xylol and then mounted permanently with mounting medium to a standard glass

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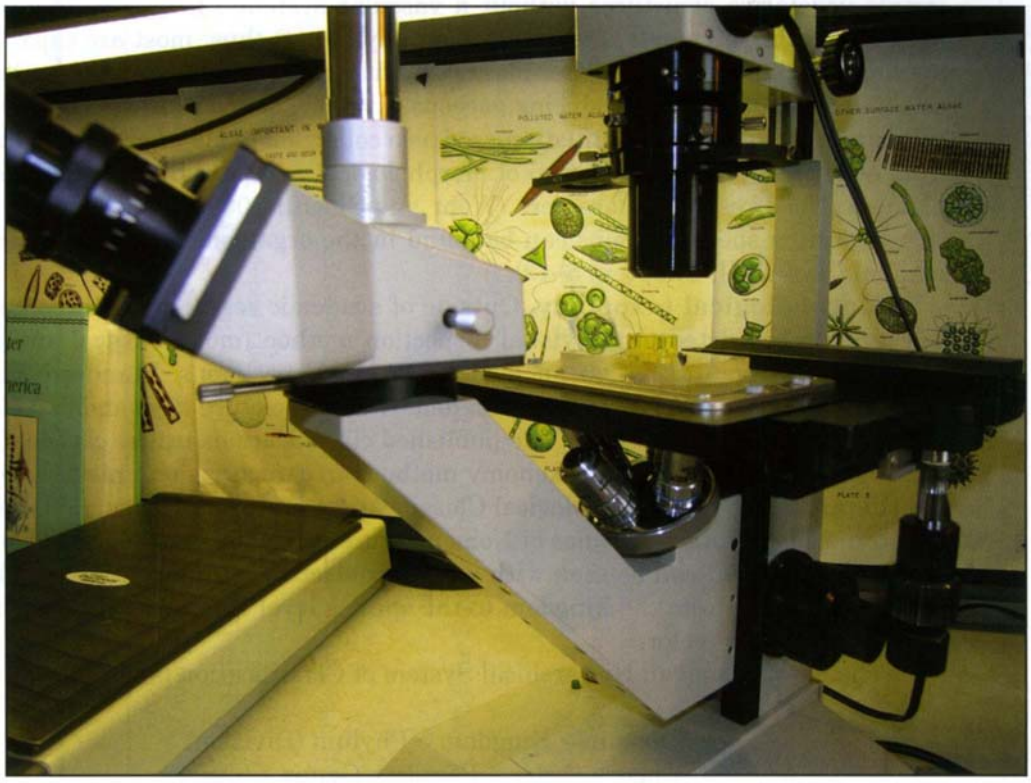

Figure 2-16 Direct examination of 5-mL Utermöhl chamber (Courtesy of Spirogyra Diversified Environmental Services, Burlington, N.C.)

slide (APHA et al. 2005, St. Amand 1997). Such mounts are suitable for examination at high magnification (1,000×) with an oil-immersion microscope objective.

Diatom mounts. Diatom taxonomy at the species level is based largely on differentiation of the intricate patterns of ornamentation on their silica cell walls (frustules). The resolution necessary for this differentiation requires high magnification and the use of oil-immersion objectives. Most high-magnification examinations are performed on thin wet mounts, permanent membrane filter mounts, or permanent diatom mounts. For unimpeded viewing of frustule ornamentation, interfering residues, such as organic cell contents, must be removed from the diatom sample before fixation into a permanent mount. Thus, a diatom mount requires special preparation. Because the silica cell walls are highly resistant, they can withstand harsh chemical treatment. Cellular organic matter is digested with concentrated nitric acid and potassium dichromate or hydrogen peroxide. Burn mounting is an alternative cleaning method, but the nitric acid method is recommended (Acker et al. 2003a, St. Amand 1997). The cleaned frustules must be washed with distilled water prior to being placed on a standard glass slide for permanent mounting (APHA et al. 2005, Acker et al. 2003b).

Identification

The organisms we think of as algae are not a natural group in terms of their genetic relatedness. They are, rather, a loose group of largely aquatic organisms with a few unifying characteristics: (1) though they exhibit a wide range of reproductive complexity, they all lack the type of reproductive structures that set apart higher plants and animals, that is, gametangia lined with nonfertile (sterile) cells; (2) they have

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simple vegetative structures without a vascular system; (3) they generally contain light-harvesting pigments, including chlorophyll *a*; (4) thus, most are capable of conducting photosynthesis as higher plants do, with photosystems I and II, utilizing H_2O as an electron donor and producing gaseous oxygen. Though they are not phylogenetically unified, the algae are still discussed as a collection of major groups distinguished from each other by a combination of morphological and physiological (photosynthetic pigments, food reserve) characters (Wehr and Sheath 2003). More detailed information regarding specific groups can be found in the organism-specific chapters in this manual (Section II, Chapters 5–13).

Morphological taxonomy. Outside of academic research facilities, most algal workers are restricted to the visual inspection method (morphological analysis with a light microscope) to assign an identity to algal specimens. Standard methods of identifying algae generally still rely on dendritic and picture keys and descriptions in published scholarly references. The published classifications utilize classical morphological characters and chemotaxonomy methods to categorize organisms according to the Hierarchical System of Biological Classification, and assign them names according to International Botanical Rules of Nomenclature. Morphological taxonomy uses diagnostic features that can be seen with a light or electron microscope. Original systematic classification from the kingdom to the species level was based on these features, including apparent color.

The basic Linnaean Hierarchical System of Classification is as follows:

Domain > Kingdom > Phylum (Division) >
Class > Order > Family > Genus > Species

Organism names are binomial, consisting of the genus and species name. Standard taxonomic references are organized according to systematic schemes based on the above hierarchy. Often, algae are discussed in terms of major functional groups. Traditionally, the term "major algal group" has been equivalent to the phylum or division level of the Hierarchical System of Biological Classification. The recognized number of divisions can range from 8 to 13. However, it can be seen from Tables 2-6 and 2-7 that there is considerable disagreement among phycologists on the definitions of major algal groups (Graham and Wilcox 2000; Cavalier-Smith 1998, 2004; Bhattacharya et al. 2004; Woese et al. 1990). For example, Wehr and Sheath (2003) have included several algal classes in the list of major groups. These "new" divisions are the result of improved visualization technology, namely electron microscopy and molecular phylogenetic analyses. It is evident that the greatest uncertainty remaining in algal taxonomy lies in the classification of the eukaryotes traditionally known as protists (see Table 2-6) (Adl et al. 2005, Parfrey et al. 2006, Burki et al. 2007).

The group known as the blue-green algae has traditionally been assigned to a botanical phylum, the Cyanophyta, because of the presence of chlorophyll *a* and their plantlike mode of photosynthesis (oxyphototrophic). More recently, this phylum has been referred to as Cyanobacteria, since molecular phylogenetic methods have confirmed that the cyanoprokaryotic organisms are genetically bacteria (Komarek and Anagnostidis 2005). In fact, there is a movement by some researchers to reclassify the cyanobacteria according to International Microbiological Nomenclature.

Algal systematics (taxonomic relationships) are continuously being revised and refined through ultrastructural analyses (electron microscopy) and use of molecular genetic techniques, such as polymerase chain reaction (PCR) and DNA sequence analysis. Taxonomists are continually reevaluating algal nomenclature based on newly elucidated phylogenetic relationships, and there are ongoing disagreements on how strictly to define groupings at every taxonomic level. It is important to be aware of

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Table 2-6 Comparisons of algal classification schemes (major groups)

Whitford and Schumacher 1984	Woese et al. 1990	Cavalier-Smith 1998, 2004	Graham and Wilcox 2000	Bhattacharya et al. 2004
Cyanophyta (Blue-Green)	Domain Bacteria; Phylum Cyanobacteria	Empire Prokaryota; Kingdom Bacteria; Subkingdom Cyanobacteria	Phylum Cyanobacteria (Chloroxybacteria)	
Rhodophyta (Red)	Domain Eukarya; Kingdom Plantae; Division Rhodophyta	Empire Eukaryota; Kingdom Plantae; Subkingdom Biliphyta; Phylum Rhodophyta	Phylum Rhodophyta	Domain Eukarya; Kingdom Plantae; Division Rhodophyta
Chlorophyta (Green)	Domain Eukarya; Kingdom Plantae; Subkingdom Chlorobionta	Empire Eukaryota; Kingdom Plantae; Subkingdom Viriplantae; Phylum Chlorophyta	Phylum Chlorophyta	Domain Eukarya; Kingdom Plantae; Phylum Viridiplantae
Euglenophyta (Euglenoid)	Domain Eukarya	Empire Eukaryota; Subkingdom Biciliata; infrakingdom Excavata; Phylum Euglenozoa	Phylum Euglenophyta	Domain Eukarya; Kingdom Cabozoa; Phylum Euglenozoa
Xanthophyta (Yellow-green)	Domain Eukarya	Empire Eukaryota; Kingdom Chromista; Subkingdom Chromobiota; infrakingdom Heterokonta; Phylum Ochrophyta; Classes Eustigmatophyceae, Raphidiphyceae, Xanthophyceae	Phylum Ochrophyta; Class Eustigmatophyceae	Domain Eukarya; Superkingdom Chromalveolata; Kingdom Chromista; Phylum Heterokontophyta
Chrysophyta (Golden) (Chrysophyceae)	Domain Eukarya	Empire Eukaryota; Kingdom Chromista; Subkingdom Chromobiota; infrakingdom Heterokonta; Phylum Ochrophyta; Class Chrysophyceae	Phylum Ochrophyta; Class Chrysophyceae	Domain Eukarya; Superkingdom Chromalveolata; Kingdom Chromista; Phylum Heterokontophyta, Stramenopiles
Chrysophyta (Golden) (Haptophytes)	Domain Eukarya	Empire Eukaryota; Kingdom Chromista; Subkingdom Chromobiota; infrakingdom Haptista; Phylum Haptophyta	Phylum Haptophyta	Domain Eukarya; Superkingdom Chromalveolata; Kingdom Chromista; Phylum Heterokontophyta; Class Haptophyceae

Table continued next page

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Table 2-6 Comparisons of algal classification schemes (major groups) (continued)

Whitford and Schumacher 1984	Woese et al. 1990	Cavalier-Smith 1998, 2004	Graham and Wilcox 2000	Bhattacharya et al. 2004
Chrysophyta (Golden) (Synurophyceae)	Domain Eukarya	Empire Eukaryota; Kingdom Chromista; Subkingdom Chromobiota; infrakingdom Heterokonta; Phylum Ochrophyta; Class Synurophyceae	Phylum Ochrophyta; Class Synurophyceae	Domain Eukarya; Superkingdom Chromalveolata; Kingdom Chromista; Phylum Heterokontophyta; Class Synurophyceae
Chrysophyta (Golden Diatoms)	Domain Eukarya	Empire Eukaryota; Kingdom Chromista; Subkingdom Chromobiota; infrakingdom Heterokonta; Phylum Ochrophyta; Class Bacillariophyceae	Phylum Ochrophyta; Class Bacillariophyceae	Domain Eukarya; Superkingdom Chromalveolata; Kingdom Chromista; Phylum Heterokontophyta (Stramenopiles)
Pyrrhophyta (Dinoflagellates	Domain Eukarya	Empire Eukaryota; Kingdom Protozoa; infrakingdom Alveolata; Phylum Myzozoa; Class Dinophyceae	Phylum Dinophyta	Domain Eukarya; Superkingdom Chromalveolata; Phylum Alveolata; Class Dinophyceae
Cryptophyta	Domain Eukarya	Empire Eukaryota; Kingdom Chromista; Subkingdom Cryptista; Phylum Cryptista; Class Cryptophyceae	Phylum Cryptophyta	Domain Eukarya; Superkingdom Chromalveolata; Kingdom Chromista; Phylum Cryptophyta
Phaeophyta (Marine)	Domain Eukarya	Empire Eukaryota; Kingdom Chromista; Subkingdom Chromobiota; infrakingdom Heterokonta; Phylum Ochrophyta; Class Phaeophyceae	Phylum Ochrophyta; Class Phaeophyceae	Domain Eukarya; Superkingdom Chromalveolata; Kingdom Chromista; Phylum Heterokontophyta (Stramenopiles)
	Domain Eukarya; Kingdom Plantae; Division Glaucophyta	Empire Eukaryota; Kingdom Plantae; Subkingdom Biliphyta; Phylum Glaucophyta	Phylum Glaucophyta,	Domain Eukarya; Kingdom Plantae; Class Glaucocystophyceae

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Table 2-7 Chemotaxonomy of major algal groups

Classical Divisions	Wehr and Sheath 2003	Photosynthetic Pigments	Starchlike Reserve
Blue-Green Algae	Cyanobacteria	Chl*, PE, PC, APC	Cyanophycean
Red Algae	Rhodophyta	Chl*, PE, PC, APC	Floridean
Green Algae	Chlorophyta	Chl*,†	True Starch
Euglenoids	Euglenophyta	Chl[a],†	Paramylon
Yellow-Green Algae	Tribophyceae, Eustigmatophyceae, Raphidophyceae	Chl[a],‡	Chrysolaminarin
Golden Algae	Chrysophyceae	Chl*,‡, fucoxanthin	Chrysolaminarin
Golden Algae	Haptophyceae	Chl*,‡, fucoxanthin	Chrysolaminarin
Golden Algae	Synurophyceae	Chl*,‡, fucoxanthin	Chrysolaminarin
Golden Algae	Bacillariophyceae	Chl*,‡, fucoxanthin	Chrysolaminarin
Dinoflagellates	Pyrrhophyta	Chl*,‡, fucoxanthin or peridinin	True Starch
Cryptomonads	Cryptophyta	Chl*,‡, PC or PE	True Starch
Brown Algae (Marine)	Phaeophyceae	Chl*,‡, fucoxanthin	Laminarin

Source: Various phycology textbooks (e.g., Sze 1998, Graham and Wilcox 2000).
*Chl = chlorophyll *a* (green); PE = phycoerythrin (red); PC = phycocyanin (blue); APC = allophycocyanin (blue); fucoxanthin and peridinin (golden to brown).
†All of the reserves are polymers of glucose. They differ by their linkages: cyanophycean and floridean α1, 4 and α1, 6 branches; true starch with amylose α1, 4 and amylopectin α1, 4 and α1, 6 branches; paramylon β1, 3; chrysolamin and laminarin β1, 3 and β1, 6 branches. Only true starch stains positively with iodine (purple to black).
‡Pellicle and periplast within plasma membrane; the rest are external to it.
(Footnote source: Wehr and Sheath 2003)

developments in algal taxonomy by reviewing technical journals such as the *Journal of Phycology*. It will be difficult, perhaps impossible, to resolve the gaps between molecular, ultrastructural, and morphological taxonomy. Inevitably, the limitations of morphological taxonomy will result in some technically incorrect identifications.

The level of taxonomic identification that is feasible for a water plant program may be limited and depends on the objectives of the program, the training of the staff, and the financial resources available. Identification to the species level is always the ideal, as it provides the most information to fully characterize the algal community structure in ecological studies. This is certainly necessary for all of the species of greatest importance in terms of abundance and biomass, and all problem species. Identification to species level often provides supplemental information on environmental conditions, as the ecological requirements of individual algae (temperature, pH, ion concentrations) can vary considerably within one genus, and can be very specific (Pan et al. 1996, Potapova and Charles 2003). Moreover, identification to the species level may be critical in investigations of potentially toxic species. Toxicity within some genera (e.g., *Microcystis, Anabaena,* and *Aphanizomenon*) is well documented only for certain species (e.g., *M. aeruginosa, A. circinalis,* and *Aph. gracile*). However, identification to the species level takes considerably longer than working at more general taxonomic levels (phylum, class, or genus). It also requires a more advanced reference

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library. For many objectives of water quality studies, identification to the genus level or even major working group (division) is adequate. In fact, the first step is to assign an organism to a major group by distinguishing between prokaryote and eukaryote and recognizing growth habit, cell morphology, apparent color, presence of motility, and internal cellular organization. It is helpful to have fresh, live samples, in addition to preserved samples, in order to view natural coloration and presence of flagella. Lugol's iodine reacts with starch and, thus, stains starch-storing taxa blue or black.

Some taxa (generic categories used for classification of organisms), notably the diatoms, some chrysophytes, and some dinoflagellates (dinophytes), cannot be identified to the species level at the magnification used for counting. For diatoms, special preparations (diatom mounts) of the frustules (cell walls) and viewing at 1,000× is required for full identification. For some chrysophytes and dinoflagellates, electron microscopy is required for identification to the species level. It is strongly recommended that a collection of photomicrographs and permanent slide mounts be maintained for future reference, training, and information exchange. Such collections are also useful in documenting seasonal changes in shape and size in particular species (e.g., *Ceratium*). Under natural conditions, some features vary in response to environmental factors: cell size, food storage, reproductive features, pigmentation, and motility.

There are numerous published references available, a partial listing of which follows. Increasingly, there are also online reference resources available. Many taxonomists begin their reference research by utilizing pictures; they consult keys last, when they are close to a decision. For the reasons discussed previously, binomial names and hierarchical placement of taxa will inevitably vary, depending on the reference consulted. Therefore, it is essential to keep a record of the taxonomic authority and key author. Accurate notes should be kept on the collection site and methodology. Such information may aid identification and will facilitate a more informed interpretation of data. When necessary, an experienced algal taxonomist should be consulted. Most state water quality agencies employ staff phycologists, and professional phycologists are also available at commercial laboratories.

Chemotaxonomy (chemical fractionation). Chemotaxonomy relies on chemical analysis (usually by high-performance liquid chromatography [HPLC]) of photosynthetic pigments and other cellular constituents to classify organisms into major taxonomic groups (phylum/division). HPLC can be used for quantification of algal chlorophylls and their degradation products (APHA et al. 2005). The method also detects carotenoid pigments, which are also present in 90% acetone extracts, but do not fluoresce. Certain chlorophyll and carotenoid pigments, or combinations of pigments, are characteristic of each division, also known as a functional algal group. HPLC quantitation of pigments extracted from a plankton sample can roughly characterize the functional groups (phyla) present in a sample population.

A crude form of chemotaxonomy occurs when Lugol's iodine is used as a preservative and selectively colors starch-storing organisms dark blue or black.

Also, another very general form of chemotaxonomy occurs when specimens are examined with a microscope equipped with illumination and optics for detection of epifluorescence. This technique exploits the specific light-absorbing and light-emitting properties of certain photosynthetic pigments (i.e., chlorophyll *a* and phycoerythrin). When chlorophyll *a* absorbs blue light, it subsequently emits (fluoresces) red light. Similarly, when phycoerythrin absorbs green light, it fluoresces orange-red light. Thus, the technique is useful for characterizing the major algal groups containing those pigments (see Table 2-7).

Particle imaging and analysis instrumentation. Digital imaging technology, originally used by oceanographic scientists, is now being tested commercially as a means to make visual identification and enumeration of algae easier and more certain

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than with a microscope (Reilly-Matthews 2007). An example of this technique is the FlowCAM® (Fluid Imaging Technologies, Yarmouth, Maine), a particle-imaging and flow-cytometry system. The system takes high-resolution, full-color digital images of every particle and cell in a fluid sample. A suite of other analytical parameters are also collected along with each image, including count, size, length, shape, and spherical diameter. Additional advanced data, such as intensity, transparency, color, biovolume, compactness, roughness, and elongation, further characterize each particle or cell. The FlowCAM's imaging capabilities are triggered by a laser that stimulates the natural fluorescence of each algal cell (Reilly-Matthews 2007).

Some uncertainties remain, though, in a totally automated, digital system for identification and enumeration. The statistical reliability of counts obtained from unconcentrated, digitally analyzed samples is not known. The system must be able to recognize natural morphological variation within a species. Finally, the utility of the system will be limited by the size of its image library, which must be built with standard techniques and references.

Molecular probes for early detection and species-specific quantitation. Basic research is being conducted to discover genetic markers for specific algal organisms that could make it possible to reduce the need for the standard method of visual identification of algae. Researchers John W. LaClaire and Schonna Manning of the University of Texas at Austin have developed a method of rapid, multiplex PCR that allows the specific and sensitive detection of harmful algae using one particular toxin-producing algal species, *Prymnesium parvum,* as a model (Pogue 2006). Further, this technique will allow analysts to quantitate this organism in water samples (Pogue 2006). The researchers expect that development of similar approaches will extend the technique of PCR-based identification to multiple algal species in any given water sample (Pogue 2006).

Quantitation (Enumeration)

Algal identification alone rarely provides all of the information needed to satisfy the objectives of water quality studies. Some type of quantitative determination must be conducted to address both operational and trending issues. This can be done with surrogate measurements, (e.g., chlorophyll *a*, ash-free dry mass, generic particle counting, and total N and P). For phytoplankton, it is most often accomplished by direct enumeration (counting). Counting is usually performed simultaneously with identification on preserved samples. There are limits to accuracy in all methods, but there are several advantages of direct counting:

1. Estimates the number of organisms and cells present with precision >90%.
2. Eliminates errors from extraneous matter that may confound dry weight or optical density methods.
3. Eliminates the environmental and species variation of pigment estimation and particle counting methods.
4. Enables observation of cell type, health, and colony nature (Lund et al. 1958).

The basic procedure for counting algae is to quantitatively fill a counting chamber (Sedgewick–Rafter, Palmer–Maloney, hemacytometer, Utermöhl chamber) with a measured subsample of the original preserved sample. After a period of settling, the organisms are identified and the chosen counting units are enumerated (Table 2-8).

The choice of counting unit is important for the calculation of statistical precision, as explained below. The generally desired reporting unit, cells/mL, results from a count of each cell. However, cells that are organized into a colony are not independently

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Table 2-8 Standard phytoplankton counting units (APHA et al. 2005)

Enumeration Method	Counting Unit	Reporting Unit
Total cell count	One cell	Cells/mL
Natural unit count	One organism (any unicellular organism or natural colony)	Units/mL
Areal standard unit count*	400 μm^2	Units/mL

*Areal standard unit equals area of four small squares in Whipple grid at a magnification of 200×.

distributed in the sample. A more statistically valid counting unit for estimates of precision is the natural unit, which may be a single cell, filament, or colony, resulting in units/mL as the reporting unit.

Statistical basis of cell counts. The true size of the phytoplankton population of a water body cannot be known because of the limitations of the methodology (i.e., every organism cannot be counted). Thus, a small sample of the population is taken and statistics are used to evaluate the probability of the measured value lying within a certain range or confidence limits around the true value (Hötzel and Croome 1999, Lund et al. 1958). The validity of using cell counts to make inferences about algal populations is based on a statistical concept, the Poisson distribution, which was devised to describe, at least approximately, total particle count data. If the density of particles counted per unit area is high enough, it closely approximates the normal distribution (Wardlaw 1985). Thus, if its underlying assumptions are met, the Poisson distribution is useful in estimates of algal abundance because it applies to counts of particles that (a) are randomly distributed in space or time, and (b) represent a small fraction of a large population of particles (Wardlaw 1985, Lund et al. 1958, Rashash and Gallagher 1995). In theory, if a plankton organism is randomly distributed in a water body, and samples small relative to the population are removed and counted by an effective process, the counts should be distributed according to the Poisson distribution, and the variation between replicate samples should be due to random sampling errors. In reality, living organisms in their natural environments are not distributed at random but may be clustered, clumped, or aggregated. However, if an acceptable degree of random distribution of plankton can be established (i.e., agreement with the Poisson distribution), it is possible to calculate standard errors and confidence limits for a count of a single sample. Because the Poisson distribution applies to counts of particles that are independent of each other, it is necessary to count discrete natural units, as described above, in order to calculate estimates of precision. The confidence limits for the unit count can be multiplied by the mean number of cells per unit in order to obtain confidence limits for cell counts. However, it is important to remember that average cell numbers per unit are subject to large errors because of the variation in cell numbers of indeterminate colonies or filaments. To obtain an accurate cell count, the number of cells in each filament or colony must be enumerated.

The sources of error can be summarized in several groups: systematic errors of the method used, random errors of sampling and subsampling, counting errors, and converting colony counts into estimates of cell population density (Hötzel and Croome 1999, Lund et al. 1958). Systematic errors refer to the errors inherent in the method used (e.g., membrane filtration, sedimentation).

Random sampling error refers to the primary Poisson condition of random distribution of organisms in the system (body of water being sampled). This type of randomness is determined by performance of a chi-square (X^2) statistical test on a number of replicate samples. If organisms clump together, greater variation between replicates

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will be found than will be expected according to the random theoretical model, and X^2 will be significantly large. If equally spaced, variation will be less than expected, and X^2 will be significantly small. The desired result is to have X^2 show no significant departure from the Poisson distribution, in other words, H_0: $\sigma^2 = \mu$. Patchiness (spatial heterogeneity) in the distribution of phytoplankton can be tested by conducting a pilot study in which several samples can be collected at slightly different spots at one site and analyzed as replicates (Vollenweider 1969).

The Poisson distribution concept can also be applied to subsampling error. Each step in the process of subsampling from the original sample container introduces a new source of error, so the number of subsampling steps should be minimized (Hötzel and Croome 1999). The distribution of units of individual species among subsamples from the same container can be tested with a chi-square (X^2) test. Also, there may be clumping error associated with the distribution of cells over the counting chamber floor. The randomness of this distribution should be tested with a chi-square (X^2) test if counting is done on only part of the chamber floor (field or strip count).

The errors resulting from the actual enumeration of algal units are different from those due to sampling, because they depend on subjective decisions made by the analyst, for example, which colonies are alive or dead, which are inside the lines of demarcation, and how many cells are in a colony (Lund et al. 1958). Counting error (precision) must be evaluated by calculation of confidence limits for each individual taxon, not for the overall sample. The acceptable level of precision is determined according to the objectives of the study and the degree of counting effort that can be applied. Precision increases with the number of units counted. Consequently, the highest levels of precision are attainable only for the most abundant taxa. Many ecological investigations are concerned only with generations, or changes greater than 100% (Hötzel and Croome 1999, Lund et al. 1958). In these cases, accuracy of ±50% is adequate. The most commonly chosen level of precision is ±20%, and the most commonly chosen level of confidence in a count of any particular taxon is the conventional "significant" level (95%) (Hötzel and Croome 1999, Utermöhl 1958). This level can be achieved with a count of 100 natural units. Of course, the chosen precision level can be achieved only for the most abundant taxa. In fact, Willen (1976) found that about six species represent 90% or more of the total phytoplankton biomass in nutrient-rich waters. She found that a simplified method is adequate, in which 60 individuals are counted for each species, resulting in a counting error of ±26%. The counting error for a single count can be estimated with 95% confidence limits by using the simplified formula:

$$\frac{2}{\sqrt{N}} \times 100 = \text{counting error } (\pm\%)$$

where N is the number of units counted of a Poisson variable (Hötzel and Croome 1999). Note that a count of 400 units is required to reduce the counting error to ±10% (Table 2-9). Detailed discussions of the statistical basis of cell counts are provided in Wardlaw (1985), Hötzel and Croome (1999), Lund et al. (1958), and Rashash and Gallagher (1995).

In summary, if organisms are randomly distributed, a single count is sufficient to estimate their abundance (Lund et al. 1958).

Performing algal counts. As mentioned previously, phytoplankton counting and identification usually occur simultaneously. The original sample container is mixed by gentle inversion for at least 30 seconds prior to withdrawal of a subsample. Unless the membrane filtration method is used, subsamples to be enumerated must be placed into one of the specialized chambers described above that contain specific, defined volumes of liquid (i.e., hemacytometer, Palmer–Maloney, Sedgewick–Rafter, or Utermöhl). If

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Table 2-9 Accuracy of various sized counts within 95% confidence limits (Lund et al. 1958)

	Approximate 95% Confidence Limits	
Number of Organisms (Units)	Expressed as Percentage Count (%)	Range
4	±100	0–8
16	±50	8–24
100	±20	80–120
400	±10	360–440
1,600	±5	1,520–1,680
10,000	±2	9,800–10,200
40,000	±1	39,600–40,400

plankton are filtered onto a membrane, the membrane is permanently mounted on a standard microscope slide (St. Amand 1997). The filter mount and some of the chambers can be counted with an upright microscope. The Utermöhl chamber is counted with an inverted microscope. The US Geological Survey (USGS) National Water Quality Assessment (NAWQA) program protocol, in agreement with this author, prescribes that counting of all phytoplankton be done with a single high dry microscope objective (40–45× objective), unless unit density is extremely low (<5) (Acker 2003). However, several authors have suggested counting at multiple magnifications (APHA et al. 2005, Lund et al. 1958, Willen 1976, Wagner et al. 2005, Hötzel and Croome 1999). Larger, rarer taxa can be counted at lower magnification (100×, 200×). However, nanoplankton and picoplankton must be counted at higher magnifications (≥400×). Picoplankton in a membrane filter mount can be counted at 1,000× on a microscope equipped with an oil-immersion objective.

Microscope calibration procedures. Regardless of the magnification or type of microscope, the counting area must be calibrated against a standard scale. Usually, an etched glass disc, known as a Whipple ocular micrometer, grid, or reticule, is installed in the plane of the real image of an ocular (Wetzel and Likens 1991). There is an upper lens mounting in the ocular that can be unscrewed. The Whipple micrometer can then be placed on the circular diaphragm found halfway down in the ocular cylinder. The image of the specimen appears to be directly under the micrometer grid. The Whipple reticule is then calibrated against a standard scale etched into a stage micrometer. The process must be performed for each specific combination of oculars and objectives (e.g., a 10× ocular and the 10× objective, 10× ocular and 20× objective). With the 10× ocular and 10× objective, the entire Whipple grid area delimits approximately 1 mm^2 (APHA et al. 2005). After calibration, the dimensions of objects can be measured directly.

Counting techniques and apparatus. The apparatus and techniques used in quantitation of phytoplankton and periphyton are described below.

Bacterial counting cells and hemacytometers. Bacterial counting cells are precisely machined glass slides with a finely ruled grid on a counting plate and specially fitted coverslip. The coverslip rests just above the counting surface on parallel platforms, so that a space of 0.1 mm remains. With the coverslip in place, a pipette should be used to introduce a drop of sample to the chamber. With high magnification (400–450×) on an upright microscope, all forms that fall within the gridded area of the cell should be counted. The number of units counted is multiplied by a multiplication factor (10,000) determined from the volume over the gridded area of the chamber (1 mm × 1 mm × 0.1 mm). Hemacytometer counts may be subject to large statistical variability (Weber 1973, Lund et al. 1958). A high plankton density is required, and it is helpful to examine a large number of mounts per sample (APHA et al. 2005, Weber 1973).

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Palmer–Maloney cell. The Palmer–Maloney cell was especially designed for enumerating nanoplankton with a high-dry objective (40–45×). It has a circular chamber 17.9 mm in diameter and 0.4 mm deep, resulting in a volume, when covered with a coverslip, of 0.1 mL. It is most useful for counting dense samples of nanoplankton at high magnification on an upright microscope (Wetzel and Likens 1991). As with the hemacytometer, the small volume presents a statistical disadvantage, especially for enumerating phytoplankton larger than 15 μm (Weber 1973). For statistically reliable data, a plankton density ≥10 units per field is required (Wetzel and Likens 1991). An aliquot of a well-mixed sample should be pipetted into one of the 2 × 5 mm channels on either side of the circular chamber with the coverslip in place. After 10 minutes of settling, the sample should be examined under high magnification and either 20–30 randomly selected Whipple fields or two perpendicular transects that cross at the center should be counted (Wetzel and Likens 1991, Weber 1973, APHA et al. 2005). The concentration of organisms should be calculated as

$$\text{no. per mL} = \frac{C \times 1{,}000\ \text{mm}^3}{A \times D \times F}$$

Where:

C = number of organisms counted
A = area of a field (Whipple grid image), mm^2
D = depth of a field (P-M cell depth), mm
F = number of fields counted

Sedgewick–Rafter counting chamber. The Sedgewick–Rafter cell consists of a standard microscope slide on which is mounted a raised rim (brass or ceramic) that creates a chamber 50 mm long by 20 mm wide and 1 mm deep. The slide has a grid floor (1,000 mm^2) and holds a total volume of 1 mL. The S-R cell can be used with either an upright or inverted microscope. With the upright microscope, the 20× objective is generally used. The longer working distances possible with inverted microscopes allow examination with ≥40× objectives. Each Sedgewick–Rafter cell must be calibrated in order to confirm that the true volume is 1 mL ± 5%. The volume can be determined by weighing, 10 times, the cell filled with deionized water and calculating the average (Hötzel and Croome 1999). Calibration also requires determination of the cell area with a vernier caliper and depth with a micrometer (Hötzel and Croome 1999, Weber 1973).

Prior to filling, a 24-mm by 60-mm, No. 1 coverslip is placed diagonally across the cell. With a Pasteur pipette, transfer a 1-mL aliquot of either the well-mixed original sample or the concentrated or diluted subsample into the open corner of the chamber until the coverslip rotates into place. Allow the cell to stand for at least 15 minutes to permit settling. Count units in randomly selected fields or transects (strips). For a strip count, examine two to four strips the length of the cell, depending on cell density, until the minimum number of units set for each taxon has been counted (Hötzel and Croome 1999, Weber 1973). For field count, examine 10–30 random Whipple fields (Hötzel and Croome 1999, Weber 1973). Thirty fields should include 90%–95% of the species present (Hötzel and Croome 1999). Since the units do not always distribute randomly in the cell, space the transects or fields along the whole chamber in order to overcome this bias (Hötzel and Croome 1999). Count all forms that are totally or partially covered by the Whipple grid; adopt a consistent system of counting organisms that touch one of the edges (Weber 1973). For the strip count, the number of organisms is calculated by the following equation:

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$$\text{no. per mL} = \frac{C \times 1{,}000\ \text{mm}^3}{L \times D \times W \times S}$$

Where:

C = number of organisms counted
L = length of each strip (S-R cell length), mm
D = depth of a strip (S-R cell depth), mm
W = width of a strip (Whipple grid image width), mm
S = number of strips counted

For a field count, the number of organisms is calculated by the following equation:

$$\text{no. per mL} = \frac{C \times 1{,}000\ \text{mm}^3}{A \times D \times F}$$

Where:

C = number of organisms counted
A = area of a field (Whipple grid image), mm^2
D = depth of a field (P-M cell depth), mm
F = number of fields counted

Utermöhl chamber. The Utermöhl chamber is a specialized cell used both in sedimentation of samples (see Sedimentation and Sedimentation Chambers sections) and for counting, specifically with the inverted microscope (Figure 2-12). The design of the inverted microscope, with the objectives mounted below the stage, allows the shorter sizes of Utermöhl chambers to be placed directly on the stage so that sedimented plankton can be viewed and counted from below. High-magnification objectives, including oil immersion, can be used. A wide range of concentrations can be obtained by selecting different chamber heights (Figure 2-12) (Weber 1973).

The complete Utermöhl chamber is a two-part assembly that consists of a vertical cylinder (tower) and a base unit. The tower is a Plexiglas™ tube inserted through and glued permanently to a flat, Plexiglas plate, such that an open cylinder is formed. The base unit is also a flat, Plexiglas plate sized to exactly match the tower plate and containing a circular opening matching the exact diameter of the tower tube. The opening is surrounded by a platform on which is placed a circular, no. 1 coverslip (Carolina Biological Supply Co., Burlington, N.C.). A thin layer of water-insoluble grease (e.g., stopcock grease on the platform seals the coverslip), which is held in place by a retention ring, thus forming a shallow (1-mL) chamber. The lower surface of the tower base plate is coated with a thin layer of water-insoluble grease and is centered precisely over the hole of the base unit, forming a sealed assembly. To prepare the sample, the original sample should be mixed by gently inverting the container for at least 30 seconds. It is important to know the exact amount of sample being concentrated, so the volume of sample should be carefully measured with a graduated cylinder, and a graduated pipette, if necessary, during the filling process. The chamber should be filled to the top edge with a slight excess forming a convex bead just above the edge. A second circular coverslip should be slid across the top of the cylinder without entrapping air bubbles. See the section on sedimentation for guidance on settling times. After an appropriate settling time, the tower may be carefully, but quickly, slid across and off of the base unit so that the supernatant drains over a sink, leaving the concentrate in the base unit chamber. As previously mentioned, the sample may be examined directly in the base unit (NCDENR 2003), or it may be resuspended by gentle pipette suction and transferred to another type of chamber for counting (e.g., Sedgewick–Rafter; APHA et al. 2005). A decision diagram for counting phytoplankton with a Utermöhl chamber is given in Hötzel and Croome (1999).

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The Utermöhl chamber counting technique is similar to the Sedgewick–Rafter procedure (i.e., either strip or field counts can be used). Some authors suggest scanning the entire chamber floor, traversing adjacent strips until the entire floor is counted, especially for sparsely occurring larger organisms (Hötzel and Croome 1999, Lund et al. 1958, Wetzel and Likens 1991, Willen 1976, Acker 2003, APHA et al. 2005). If this technique is used, the cell count relates directly to the sample volume settled:

$$\text{no. per mL} = \frac{C}{V}$$

Where:

C = number of organisms counted
V = volume settled, mL

However, all agree that, for dense samples, a partial area of the chamber (random fields or transects) may be counted. A common procedure involves counting at least two perpendicular transects (one vertical, one horizontal) through the center of the chamber (Weber 1973, APHA et al. 2005). When central transects are used, a factor is derived from the ratio of the area of the chamber floor covered by the transects counted to the entire chamber floor (Hötzel and Croome 1999). This factor is multiplied by the number of units counted to obtain the number of units per milliliter as follows:

$$\text{strip count (no. per mL)} = \frac{C \times A_t}{L \times W \times S \times V}$$

Where:

C = number of organisms counted
A_t = total area of bottom settling chamber, mm^2
L = length of a strip, mm
W = width of a strip (Whipple grid image width), mm
S = number of strips counted
V = volume of the chamber, mL (APHA et al. 2005)

Alternatively, random field counts can be made; the number depends on the density of organisms found, but the general rule is to count a minimum of 100 of the most abundant taxon (Weber 1973). For a field count, the equation becomes

$$\text{field count (no. per mL)} = \frac{C \times A_t}{A_f \times F \times V}$$

Where:

C = number of organisms counted
A_t = total area of bottom settling chamber, mm^2
A_f = area of a field (Whipple grid image area), mm^2
F = number of fields counted
V = volume of chamber, mL (APHA et al. 2005)

Membrane filter. A 1-in., 0.45-µm filter is used with a vacuum pump and filtration apparatus (see also the Membrane Filtration section). A measured volume of the water sample should be passed through the membrane filter under a vacuum of 0.5 atm (Weber 1973). The filter should be allowed to dry at room temperature for 5 minutes and then placed on top of two drops of immersion oil on a microscope slide. Two drops of oil should be placed on top of the filter and allowed to dry clear at room temperature

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(approximately 48 hours). A coverslip should be affixed by applying a permanent mounting medium such as Naphrax™ (Northern Biological Supplies), Kleermount® (Carolina Biological Supply, Burlington, N.C.), Practamount (ASCO Laboratories, Manchester, UK), or Canada balsam (Spectrum Quality Products, Gardena, Calif.); then the organisms should be enumerated. The entire filter can be scanned, or 30 random fields can be counted. The number of fields in which each species occurred should be recorded (APHA et al. 2005). The number of organisms per milliliter can be calculated as follows:

$$\text{no. per mL} = \frac{N \times Q}{V \times D}$$

Where:

N = density (organisms/field) from Table 10200: II (APHA et al. 2005)
Q = number of fields per filter
V = milliliters filtered
D = dilution factor (0.96 for 4% formalin preservative)

Techniques for periphyton. Periphyton samples that have been suspended and mixed in liquid can be analyzed by the techniques described above for the Palmer–Maloney cell, the Sedgewick–Rafter cell, and the Utermöhl chamber. For the Sedgewick–Rafter cell, a 1-mL aliquot of the suspension should be transferred and a strip count performed as described above (APHA et al. 2005). Alternatively, a measured aliquot of the suspension can be transferred to a standardized Utermöhl chamber for examination by the inverted microscope method. After a suitable period of settling, organisms can be counted in the settling chamber within a known number of strips or random fields. The count should be expressed as cells or filaments per square milliliter of substrate area. Algal density per unit area of substrate can be calculated as follows:

$$\text{organisms/mm}^2 = \frac{N \times A_t \times V_t}{A_c \times V_s \times A_s}$$

Where:

N = number of organisms counted
A_t = total area of chamber bottom, mm^2
V_t = total volume of original suspension, mL
A_c = area counted (strips or fields), mm^2
V_s = sample volume used in chamber, mL
A_s = surface area of slide or substrate, mm^2 (APHA et al. 2005)

Determination of biomass or biovolume. Algal numerical density (cells/mL) presents only a partial picture of the phytoplankton community. Estimates of algal standing crop (biomass) are useful for many objectives, such as evaluating nutrient status of source waters, designing water treatment processes, treatment of algal blooms, and so forth. There is no easy or completely accurate way to transform cell counts into volumes of living biomass, but there are numerous accepted methods for directly or indirectly determining biomass, several of which are discussed below. The most commonly used methods involve direct quantitation of algal photosynthetic pigments or indirect estimation of biovolume (Hötzel and Croome 1999).

Biovolume. Often, data derived on a volume-to-volume basis provide a more complete characterization of the importance of various taxa in the community (APHA et al. 2005). Cell volume (biovolume) is determined because of the great differences in cell dimensions among species and sometimes seasonally or spatially within the same

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species under different growth conditions (e.g., light, nutrient limitations; Wetzel and Likens 1991). For example, in reservoirs, high levels of suspended sediments in the shallow, upper (riverine) portion inhibit light penetration in the photic zone, limiting cell growth. Consequently, reservoirs have significant horizontal gradients of light availability and, often, cell sizes of individuals within the same species. Cell volumes are calculated for each species by applying cellular dimensions to standard formulae for solid geometric shapes most closely matching the shape of the cells (e.g., spherical, cylindrical, rectangular, or cone; Wetzel and Likens 1991). Cell volume should be determined by using the simplest geometric configuration that best fits the cell being measured (APHA et al. 2005). Examples of geometric shapes are provided in Wetzel and Likens (1991), Wagner et al. (2005), and Hillebrand et al. (1999). Biovolumes are listed for selected species by Willen (1976) and Hillebrand et al. (1999). Cellular dimensions are determined with a calibrated Whipple reticule that has a subdivided central square. Optical measurements can be made for 20 representative individuals of each major species for each sampling period (Weber 1973, APHA et al. 2005). The average volume (cubic micrometers) can be determined and multiplied by the number of organisms per milliliter. Total wet algal volume can be computed as:

$$V_t = \sum_{i=1}^{n} (N_i \times V_i)$$

Where:

V_t = total plankton cell volume, mm^3/L
N_i = number of organisms of the *i*th species/L
V_i = average volume of *i*th species, μm^3 (APHA et al. 2005)

Biovolume data can be converted to an indirect estimate of fresh weight biomass by approximating cell density as 1.000. Thus, biovolume is converted from cubic micrometers per milliliter to milligrams per liter by a factor of 1×10^{-6}.

Pigment Concentrations

Determinations of the concentrations of photosynthetic pigments can be used to estimate the composite biomass of algal populations (Wetzel and Likens 1991). Chlorophyll *a* is the pigment most widely utilized for this purpose because the analytical methodology of extracting it from harvested cells is well documented and relatively direct, accurate, and inexpensive (APHA et al. 2005, USEPA 1997, ASTM 2004, Wetzel and Likens 1991, Hötzel and Croome 1999). It can be measured by spectrophotometric, fluorometric, or high-performance liquid chromatographic techniques (APHA et al. 2005). Chlorophyll *a* can also be measured *in vivo* by means of fluoroprobes when readings are standardized with extractive data (Turner Designs, Sunnyvale, Calif.; Hach Hydrolab Corp., Loveland, Colo., USA). Concentration data are then converted to an indirect estimate of biomass by approximating that chlorophyll *a* constitutes, on average, 1.5% of the dry weight of organic matter of algae (APHA et al. 2005). Thus, biomass can be estimated by multiplying chlorophyll *a* content by a factor of 67 (APHA et al. 2005). Chlorophyll *a* concentrations are subject to considerable physiological variability due to factors including metabolism, light, temperature, nutrient availability, species, and so forth. Additionally, pigments of photosynthetic and nonphotosynthetic sulfur bacteria can interfere with the analysis of chlorophyll in algae (Wetzel and Likens 1991). Further, the phaeophytin degradation products of chlorophyllous pigments can also confound spectrophotometric and fluorometric determinations of chlorophyll (Wetzel and Likens 1991). This interference can be quantified by a separate determination of phaeophytin concentration. Detailed discussions of procedures for determining

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pigments are available in *Standard Methods* (APHA et al. 2005), Wetzel and Likens (1991), USEPA (1997), Hötzel and Croome (1999), and ASTM (2004).

Other pigments that occur in algae include chlorophylls *a* and *b*, xanthophylls, phycobilins, and carotenes. Their concentrations can also be chemically determined and used to separate the major algal groups (see Chemotaxonomy section and Table 2-7). The phycobilin pigments, phycocyanin and phycoerythrin, can be determined by both extractive spectrophotometric and *in vivo* fluorometric techniques (Turner Designs; Hach Hydrolab Corp.; Prozyme®Glyko®, San Leandro, Calif.). Phycobilin pigment concentrations can provide biomass estimates specific to certain algal groups, especially cyanobacteria. Fluoroprobes can be configured to detect the fluorescence band of either phycocyanin for freshwater cyanobacteria or phycoerythrin for marine cyanobacteria (Hach Hydrolab Corp.).

Gravimetric Methods

Algal biomass can be estimated by the determination of dry weight or ash-free dry weight. Dry weight can be determined by placing 100 mg of wet concentrated sample in a clean, ignited, and tared porcelain crucible and drying at 105°C for 24 hours (APHA et al. 2005). Alternatively, a known volume of sample can be filtered through a preweighed 0.45-μm-pore-diameter membrane or a prerinsed, dried, and preweighed glass-fiber filter (be aware that the small sample used in direct filtration may lead to error) (APHA et al. 2005). The sample should be cooled in a desiccator and weighed. For ash-free dry weight, the dried sample can be ignited at 500°C for 1 hour. After it cools, the ash can be rewetted with distilled water in order to rehydrate clays, and brought to constant weight at 105°C (APHA et al. 2005). The remainder is the residual ash, which may comprise 5%–50% of algal dry weight. Gravimetric methods are not generally considered practical for natural populations of algae because of the inevitable presence of silt and particulate detritus in the water (Wetzel and Likens 1991, APHA et al. 2005). In fact, the weight of particulate detritus in freshwater greatly exceeds the weight of the algae, often constituting over 80% of suspended material (Wetzel and Likens 1991). Moreover, drying by heat results in appreciable loss of volatile organic matter.

Total particulate organic carbon. Measurement of the total particulate organic carbon in an algae sample is sometimes used to estimate algal biomass. As in the gravimetric methods described above, particulate matter is filtered from the water sample onto a glass-fiber filter (Wetzel and Likens 1991). The organic matter on the filters is then completely chemically oxidized (Wetzel and Likens 1991). The method is subject to large errors because of the inevitable inclusion of extraneous particulate detritus. Routinely analyzed TOC rarely correlates well with algal biomass. The organic carbon content of algae can be expressed as a ratio of cellular organic carbon (in μg) to cell volume ($\mu m^3/L$), approximately 0.10 (Wetzel and Likens 1991). Other approximate conversion factors have been developed, for example, 0.2 pg $C/\mu m^3$ for nondiatom algae or 0.1 pg $C/\mu m^3$ for flagellates (Wetzel and Likens 1991).

Adenosine triphosphate. Measurement of adenosine triphosphate (ATP) in algae provides the only means of determining the total *viable* algal biomass, as ATP occurs in all organisms, but only in living cells (APHA et al. 2005). Since ATP is not associated with nonliving particulate material, its measurement is a sensitive, specific means of determining biomass. The ratio of ATP to biomass varies from species to species but is constant enough to permit reliable estimates of biomass from ATP measurements (APHA et al. 2005). However, the technique requires specialized detection equipment (Turner Designs) designed specifically for measuring ATP (APHA et al. 2005). Thus, it may not be a practical technique for most water utilities.

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QUALITY ASSURANCE

Each of the topics covered in this chapter, from site and equipment selection through sample analysis, represents an essential element of the quality system for algal monitoring, that is, the activities involved in the production of data of known accuracy and precision (Garfield 1991, Hötzel and Croome 1999). A quality assurance program formally identifies all of the elements of an operation that impact the quality of data and institutes systematic plans (quality assurance/quality control [QA/QC]) to ensure that data meet established standards. Quality assurance activities are differentiated from quality control activities (Garfield 1991, ISO/IEC 17025:2005). It is helpful to think of the quality assurance activities as the planned processes designed to produce acceptable results in a systematic, reliable fashion (ISO/IEC 17025:2005). Areas covered by quality assurance include

1. Training of staff;
2. Providing reasonable facilities and suitable equipment;
3. Documenting analytical methods;
4. Documenting procedures for sampling, sample handling, and sample analysis;
5. Documenting procedures for data reporting and record keeping; and
6. Implementing and documenting quality control procedures (Hötzel and Croome 1999).

Quality control activities emphasize testing and rejection of data that do not meet acceptable error sizes and confidence limits (ISO/IEC 17025:2005). Quality control procedures should be implemented at critical steps in the process, for example, sample collection, subsampling from the original sample bottle, and subsampling within a counting chamber if only part of the floor is counted (Hötzel and Croome 1999). Also, laboratory and operator precision can be tested. To test individual operator error, Hötzel and Croome (1999) suggest that a count on three subsamples of one sample be conducted every three months, with errors and confidence limits calculated. To test for precision within the laboratory, Hötzel and Croome (1999) suggest that every six months a single set of three subsamples of a single sample be counted by each operator, with the error and confidence limits calculated. External audits are also recommended at regular (six-month) intervals. It is recommended that 5% of samples be recounted by an outside laboratory (APHA et al. 2005).

In summary, quality assurance is about improving or stabilizing processes to avoid or minimize unacceptable results. It does not eliminate the need for quality control testing (ISO/IEC 17025:2005). The International Organization for Standardization has issued the main quality assurance standard for testing and calibration laboratories (ISO/IEC 17025:2005). Other detailed discussions of QA/QC procedures are presented in Hötzel and Croome (1999) and Garfield (1991).

REFERENCES AND BIBLIOGRAPHY

Acker, F. 2003. Analysis of USGS NAWQA program phytoplankton samples. Protocol P-13-52. In: *Protocols for the analysis of algal samples collected as part of the US Geological Survey National Water-Quality Assessment Program.* D.F. Charles, C. Knowles, R.S. Davis, eds. Philadelphia: The Academy of Natural Sciences, Patrick Center for Environmental Research–Phycology Section.

Acker, F., B. Russell, and E. Hagan. 2003a. Diatom cleaning by nitric acid digestion with microwave apparatus. Protocol P-13-42. In: *Protocols for the analysis of algal samples collected as part of the US Geological Survey National Water-Quality Assessment Program.* D.F. Charles, C. Knowles, R.S. Davis, eds. Philadelphia: The Academy of Natural Sciences, Patrick Center for Environmental Research–Phycology Section.

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Acker, F., B. Russell, and E. Morales. 2003b. Preparation of diatom slides using Naphrax™ mounting medium. Protocol P-13-49. In: *Protocols for the analysis of algal samples collected as part of the US Geological Survey National Water-Quality Assessment Program.* D.F. Charles, C. Knowles, R.S. Davis, eds. Philadelphia: The Academy of Natural Sciences, Patrick Center for Environmental Research–Phycology Section.

Adl, S.M., A.G.B. Simpson, M.A. Farmer, R.A. Anderson, O.R. Anderson, J.R. Barta, S.S. Bowser, G. Brugerolle, R.A. Fensome, S. Fredericq, T.Y. James, S. Karpov, P. Kugrens, J. Krug, C.E. Lane, L.A. Lewis, J. Lodge, J. Lynn, D.H. Mann, D.G. McCourt, R.M. Mendoza, L. Moestrup, S.E. Mozeley-Standridge, T.A. Nerad, C.A. Shearer, A.V. Smirnov, F.W. Spiegel, and M.F.J.R. Taylor. 2005. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *J. Eukaryotic Microbiol*, 52:399–451.

American Public Health Association (APHA), American Water Works Association (AWWA), Water Environment Federation (WEF). 2005. *Standard methods for the examination of water and wastewater.* 21st ed. Washington, D.C.: APHA.

ASTM International. 2004. Standard practices for measurement of chlorophyll content of algae in surface waters. D 3731-87 (2004). In: *Annual book of ASTM standards.* West Conshohocken, Pa.: ASTM International.

Bhattacharya, D., H.S. Yoon, and J.D. Hackett. 2004. Photosynthetic eukaryotes unite: Endosymbiosis connects the dots. *BioEssays*, 26(1): 50–60. Baker, P.D., and L.D. Fabro. 2002. A guide to the identification of common blue-green algae (cyanoprokaryotes) in Australian freshwaters. Identification and ecology guide No. 25. 2nd ed. Canberra, Australia: Cooperative Research Centre for Freshwater Ecology.

Bourelly, P. 1966. Les Algues d'Eau Douce. Tome I. Les Algues Vertes. Paris: N. Boubée et Cie.

Bourelly, P. 1968. Les Algues d'Eau Douce. Tome II. Les Algues Jaunes et Brunes, Chrysophycées, Pheophycées, Xanthyophycées, et Diatomées. Paris: N. Boubée et Cie.

Bourelly, P. 1970. *Les Algues d'Eau Douce.* Tome III. Les Algues Bleues et Rouges, Les Eugléniens, Peridiniens et Cryptomonadines. Paris: N. Boubée et Cie.

Bukata, R.P., J.H. Jerome, and J.E. Bruton. 1988. Relationship among Secchi disk depth, beam attenuation coefficient, and irradiance attenuation coefficient for Great Lakes waters. *Jour. Great Lakes Research* 14:3:347–355.

Burki, F., K. Shalchian-Tabrizi, M. Minge, A. Skjaeveland, S.I. Nikolaev, K.S. Jakobsen, and J. Pawlowski. 2007. Phylogenomics reshuffles the eukaryotic supergroups. *PLoS ONE* 2 (8:e790):e790. doi:10.1371/journal. Pone.0000790.

Campbell, P.H. 1973. Studies on brackish water phytoplankton. Sea Grant Publication, UNC-SG-73-07. Chapel Hill: University of North Carolina.

Canter-Lund, K., and J.W.G. Lund. 1996. *Freshwater algae: Their microscopic world explored.* Bristol, U.K.: Biopress Ltd.

Carolina Biological Supply Company. 2000. Material safety data sheet. Technical document. Burlington, N.C.: Carolina Biological Supply Company.

Cavalier-Smith, T. 1998. A revised six-kingdom system of life. *Biol. Rev.*, 73:203–266.

Cavalier-Smith, T. 2004. Only six kingdoms of life. *Proc. R. Soc. Lond.*, B (2004) 271:1251–1262.

Chapman, A. 2002. Identification of common planktonic freshwater cyanobacteria (blue-green algae) with an emphasis on nuisance and toxin producing taxa. Cyanobacteria Workshop. Prepared and presented at the Florida Lake Management Society 2002 Annual Conference Workshop, June 10, 2002. Palatka, Fla: Greenwater Labs/Cyanolab.

Cocke, E.C. 1967. *The myxophyceae of North Carolina.* Ann Arbor, Mich.: Edwards Brothers.

Cox, E.J. 1996. *Identification of freshwater diatoms from live material.* London: Chapman & Hall.

Croasdale, H.T., C.E.M. Bicudo, and G.W. Prescott. 1983. *A synopsis of North American desmids.* Part II. Desmidiaceae: Placodermae, Section 5. Lincoln: University of Nebraska Press.

Dillard, G.E. 1989a. *Bibliotheca phycologia.* Band 81. *Freshwater algae of the southeastern United States.* Part 1. Chlorophyceae: Volvocales, Tetrasporales and Chlorococcales. Stuttgart: J. Cramer.

Dillard, G.E. 1989b. *Bibliotheca phycologia.* Band 83. *Freshwater algae of the southeastern United States.* Part 2. Chlorophyceae: Ulotrichales, Microsporales, Cylindrocapsales, Sphaeropleales, Chaetophorales, Cladophorales, Schizogoniales, Siphonales and Oedogoniales. Stuttgart: J. Cramer.

Dillard, G.E. 1990. *Bibliotheca phycologica.* Band 85. *Freshwater algae of the southeastern United States.* Part 3. Chlorophyceae:

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Zygnematales: Zygnemataceae, Mesotaeniaceae, and Desmidiaceae (Section 1). Stuttgart: J. Cramer.

Dillard, G.E. 1991a. *Bibliotheca phycologica.* Band 89. *Freshwater algae of the southeastern United States.* Part 4. Chlorophyceae: Zygnematales: Desmidiaceae (Section 2). Stuttgart: J. Cramer.

Dillard, G.E. 1991b. *Bibliotheca phycologica.* Band 90. *Freshwater algae of the southeastern United States.* Part 5. Chlorophyceae: Zynematales: Desmidiaceae (Section 3). Stuttgart: J. Cramer.

Dillard, G.E. 1993. *Bibliotheca phycologica.* Band 93. *Freshwater algae of the southeastern United States.* Part 6. Chlorophyceae: Zygnematales: Desmidiaceae (Section 4). Stuttgart: J. Cramer.

Fawley, M.W., M.L. Dean, S.K. Dimmer, and K.P. Fawley. 2005. Evaluating the morphospecies concept in the Selenastraceae (Chlorophyceae, Chlorophyta). *J. Phycol.*, 42(1): 142–154.

Furet, J.E., and K. Benson-Evans. 1982. An evaluation of the time required to obtain complete sedimentation of fixed algal particles prior to enumeration. *Br. Phycol. J.*, 17:253–258.

Garfield, F.M. 1991. *Quality assurance principles for analytical laboratories.* Arlington, Va.: Association of Official Analytical Chemists.

Graham, L.E., and L. Wilcox. 2000. *Algae.* Upper Saddle River, N.J.: Prentice Hall.

Hasle, G.R. 1978. The inverted-microscope method. Chapter 5.2.1 in *Phytoplankton manual,* A. Sournia, ed. Paris: United Nations Educational, Scientific, and Cultural Organization.

Hillebrand, H., C-D Dürselen, D. Kirschtel, U. Pollingher, and T. Zohary. 1999. Biovolume calculation for pelagic and benthic microalgae. *J. Phycol.*, 35:403–424.

Hötzel, G., and R. Croome. 1999. A phytoplankton methods manual for Australian Freshwaters. LWRRDC Occasional Paper 22/99. Canberra, Australia: Land and Water Resources Research Development Corporation, http://downloads.lwa2.com/downloads/publications_pdf/PR990300.pdf

Huber-Pestallozzi, G. 1983. Das Phytoplankton des Suesswassers, Systematik und Biologie. *Die Binnengewaesser,* Band XVI, Teil 1–8. Stuttgart: E.Schweizerbart'sche Verlagsbuchhandlung.

Husar, V.L.M. 1996. Planktonic algae, other than desmids, of three Amazonian systems. *Amazoniana,* 14:37–73.

International Organization for Standardization (ISO). 2005. ISO/IEC 17025:2005. General requirements for the competence of testing and calibration laboratories. Geneva, Switzerland: ISO.

Kim, Y.C. 1967. The Desmidiaceae and Mesotaeniaceae in North Carolina. PhD diss., North Carolina State University.

Klaveness, D. 1985. Classical and modern criteria for determining species of Cryptophyceae. *Bulletin of Plankton Society of Japan,* 32:111–128.

Komarek, J., and K. Anagnostidis. 1998. Cyanoprokaryota I. Chroococcales, in *Süßwasserflora von Mitteleuropa,* Band 19, Koeltz. Koenigstein: Scientific Books.

Komarek, J., and K. Anagnostidis. 2005. Cyanoprokaryota II. Oscillatoriales, in *Süßwasserflora von Mitteleuropa,* Band 19/2, Koeltz. Koenigstein: Scientific Books.

Leedale, G.F. 1967. *Euglenoid flagellates.* Englewood Cliffs, N.J.: Prentice Hall.

LI-COR Inc. 2005. LI-COR terrestrial radiation sensors: instruction manual. Lincoln, Neb.: LI-COR Inc. 27 pp.

Lund, J.W.C., C. Kipling, and E.D. LeCren. 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimating by counting. *Hydrobiologia,* 11(2): 143–170.

Martin, J.L. 1999. *Marine biodiversity monitoring: Protocol for monitoring phytoplankton.* Report by the Marine Biodiversity Monitoring Committee (Atlantic Maritime Ecological Science Cooperative, Huntsman Marine Science Center) to the Ecological Monitoring and Assessment Network of Environment Canada. New Brunswick, Canada: Department of Fisheries and Oceans, Biological Station, St. Andrews.

North Carolina Department of Environment and Natural Resources (NCDENR). 2003. Standard operating procedure for algae and aquatic plant sampling and analysis. Technical document. Raleigh, N.C.: NCDENR, Division of Water Quality, Water Quality Section, Environmental Sciences Branch.

Pan, Y., R.J. Stevenson, B.H. Hill, A.T. Herlihy, and G.B. Collins. 1996. Using diatoms as indicators of ecological conditions in lotic systems: A regional assessment. *J. N. Am. Benthol. Soc.,* 15(4): 481–495.

Parfrey, L.W., E. Barbero, E. Lasser, M. Dunthorn, D. Bhattacharya, D.J. Patterson, and L.A. Katz. 2006. Evaluating support for the current classification of eukaryotic diversity. *PLoS Genet.* 2(12):e220. Epub 2006 Nov 13.

Patrick, R. and C.W. Reimer. 1975. *The diatoms of the United States,* Vol. 2. Philadelphia: Academy of Natural Sciences.

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Patrick, R., and C.W. Reimer. 1966. *The diatoms of the United States*, Vol. 1. Philadelphia: Academy of Natural Sciences.

Pogue, G. 2006. Molecular probes for early detection and species specific quantitation. E-mail communication.

Porter, S.D., T.F. Cuffney, M.E. Gurtz, and M.R. Meador. 1993. Methods for collecting algal samples as part of the National Water Quality Assessment Program. US Geological Survey Open-File Report 93-409. Raleigh, N.C.: USGS, http://water.usgs.gov/nawqa_home.html.

Potapova, M., and D.F. Charles. 2003. Distribution of benthic diatoms in US Rivers in relation to conductivity and ionic composition. *Freshwater Biology*, 48:1311–1328.

Prescott, G.W. 1973. *Algae of the western Great Lakes Area.* Dubuque, Iowa: Wm. C. Brown Co.

Prescott, G.W. 1978. *How to know the freshwater algae.* Dubuque, Iowa: Wm. C. Brown Co.

Prescott, G.W., H.T. Croasdale, and W.C. Vinyard. 1975. *A synopsis of North American desmids.* Part II. Desmidiaceae: Placodermae, Section 1. Lincoln: University of Nebraska Press.

Prescott, G.W., H.T. Croasdale, and W.C. Vinyard. 1977. *A synopsis of North American desmids.* Part II. Desmidiaceae: Placodermae, Section 2. Lincoln: University of Nebraska Press.

Prescott, G.W., H.T. Croasdale, W.C. Vinyard, and C.E.M. Bicudo. 1981. *A synopsis of North American desmids.* Part II. Desmidiaceae: Placodermae, Section 4. Lincoln: University of Nebraska Press.

Rashash, D.M.C. and D.L. Gallagher. 1995. An evaluation of algal enumeration. *Jour AWWA* 87:4:127.

Reilly-Matthews, B. 2007. Particle imaging and analysis instrumentation minimizes taste and odor complaints. *Jour AWWA* 98(11): 50.

Round, F.E., R.M. Crawford, and D.G. Mann. 1990. *The Diatoms.* New York: Cambridge University Press.

Schwoerbel, J. 1970. *Methods of hydrobiology: freshwater biology.* New York: Pergamon Press.

Smith, G.M. 1950. *The freshwater algae of the United States.* New York: McGraw-Hill.

Sournia, A. 1978. *Phytoplankton manual.* Monogr. Oceanogr. Methodol. No. 6. Paris: United Nations Educational, Scientific & Cultural Org.

St. Amand, A.L. 1997. Protocol for making permanent algal mounts using HPMA. Technical Document. St. Joseph, Mich.: PhycoTech.

Sze, P. 1998. *A biology of the algae.* 3rd ed. Boston: McGraw-Hill. 278 p.

Tiffany, L.H. 1934. *The plankton algae of the west end of Lake Erie.* Ohio State University Contribution No. 6. Columbus: Ohio State University Press.

US Environmental Protection Agency (USEPA). 1997. Method 445.0, *In vitro* determination of chlorophyll a and phaeophytin a in marine and freshwater algae by fluorescence. Revision 1.2, September, 1997. Cincinnatti, Ohio: USEPA, National Exposure Research Laboratory, Office of Research and Development, 445.0-1, http://www.epa.gov/nerlcwww/m445_0.pdf.

Utermöhl, H. 1958. Zur Vervollkommung der quantitative Phytoplankton Methodik (Toward a perfection of quantitative phytoplankton methodology), *Mitteilungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie*, 9, 1-38.

Vanormelingen, P., E. Hegewald, A. Braband, M. Kitschke, T. Friedl, K. Sabbe, and W. Vyverman. 2007. The systematics of a small spineless desmodesmus species, D. costato-granulatus (Sphaeropleales, Chlorophyceae) Based on ITS2 rDNA Sequence Analyses and Cell Wall Morphology. *J. Phycol.*, 43:378–396.

Vinyard, W. 1979. *Diatoms of North America.* Eureka, Calif.: Mad River Press.

Vollenweider, R.A. 1969. *A Manual on Methods for Measuring Primary Production in Aquatic Environments.* IBP Handbook No. 12. Oxford, UK: Blackwell Scientific Publ.

Wagner, K.J., A. St. Amand, and L.C. Ehrlich. 2005. Taxonomy, ecology and control of nuisance algae. Seminar Handbook. Prepared for presentation at: Algal Taxonomy Workshop, NALMS 14th Annual Southeastern Lakes Management Conference, Asheville, NC, April 13–15, 2005, North Carolina Lake Management Society.

Wardlaw, A.C. 1985. *Practical statistics for experimental biologists.* New York: John Wiley & Sons.

Weber, C.I. 1971. A *guide to the common diatoms at water pollution surveillance system stations.* Cincinnati, Ohio: USEPA.

Weber, C.I. 1973. Biological field and laboratory methods for measuring the quality of surface waters and effluents. EPA-670/4-73-001. Cincinnati, Ohio: USEPA, National Environmental Research Center, Office of Research & Development.

Wehr, J.D., and R.G. Sheath. 2003. *Freshwater algae of North America: Ecology and classification.* New York: Elsevier Academic Press.

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Wetzel, R.G. 2001. *Limnology: Lake and river ecosystems.* 3rd ed. New York: Elsevier Academic Press.

Wetzel, R.G., and G.E. Likens. 1991. *Limnological analyses.* 2nd ed. New York: Springer-Verlag.

Whitford, L.A. 1974. Additions to the freshwater algae in North Carolina, VIII. *Journal of the Elisha Mitchell Sci. Soc.*, 90:113–117.

Whitford, L.A. 1982. Additions to the freshwater algae in North Carolina, X. *Journal of the Elisha Mitchell Sci. Soc,.* 98:32–36.

Whitford, L.A., and G.J. Schumacher. 1984. *A manual of fresh-water algae.* Raleigh, N.C.: Sparks Press.

Wildlife Supply Company®. 2007. Catalog. Buffalo, New York.

Willen, E. 1976. A simplified method of phytoplankton counting. *Br. Phycol. J.*, 11:265–278.

Woese, C.R., O. Kander, and M.L. Wheelis. 1990. Toward a natural system of organisms: Proposal for the domains Archea, Bacteria, and Eukarya. *Proceedings of the National Academy of Sciences,* 87:4576–4579.

Selected Algal Taxonomic Sources

Balogh Scientific Books (USA)
1911 North Duncan Road
Champaign, IL 61821
217-355-9331
balogh@balogh.com

Lubrecht & Cramer Ltd. (USA)
R.D. 1, Box 244
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Forestburgh, NY 12777

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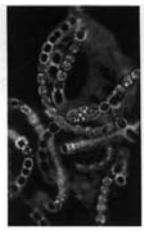

Chapter 3

Detection of Cyanotoxins During Potable Water Treatment

Judy A. Westrick, Benjamin Southwell, David Szlag, and Paul V. Zimba

In 2001, the United States Environmental Protection Agency (USEPA) listed cyanotoxins as priority contaminants in potable waters; these include the hepatotoxin microcystin, the cytotoxin cylindrospermopsin, and the neurotoxin anatoxin-a. It is critical to appreciate that commonly either a cyanobacterium or a cyanobacterial bloom can produce multiple toxins simultaneously (e.g., lipopolysaccharide toxins are found in all cyanobacteria in addition to USEPA-listed toxins, and/or simultaneous production of anatoxin-a and microcystins has been observed) (Park et al. 1993). Synergistic effects of multiple cyanotoxins present may also increase the likelihood of adverse reaction/response to toxin ingestion as known from toxicological studies (Ecobichon 1997). Therefore simultaneous assessment by either individual analyses or a single method analyzing for multiple toxins is essential for accurate identification of cyanotoxins present in samples (Park et al. 1993).

The potable water industry has two types of detection methodologies that can be used: screening assays and analytical methods for identification and quantification of cyanotoxins. Herein sample preparation, screening assays, analytical methods, and genomic/proteomic techniques are reviewed, as shown in Figure 3-1.

Methodologies for assessing the presence of these compounds will be discussed, with recommendations regarding methodologies found effective in the personal experience of the authors and from review of the literature. The Sample Preparation section includes the concentration and extraction of intracellular, extracellular, and total cyanotoxins. The Screening Assays section discusses the methodology, sensitivity, and selectivity of these assays. The Analytical Techniques section discusses techniques that have been used to identify and quantify the priority toxins, focusing on liquid

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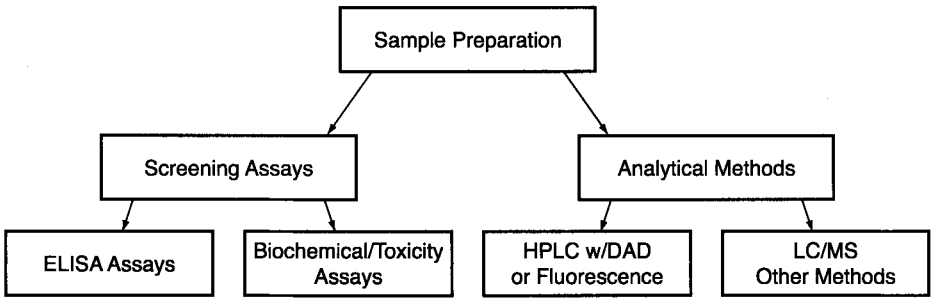

Figure 3-1 Schematic of cyanotoxin methodology

chromatography with various detectors. Liquid chromatography is the most common methodology used by commercial and utility laboratories to quantify cyanotoxins.

CYANOTOXIN STANDARDS

The commercial availability of the priority algal toxins is limited. Over the last couple of years it has been easier to purchase microcystin LR and RR, anatoxin-a, and cylindrospermopsin; however, it is still difficult to purchase microcystin LA and YR. Many available toxins are not certified, leading to issues of purity and guaranteed quantity. In the past year, a certified standard at 10 µg/mL of microcystin-LR (Sigma, USA) has become commercially available. A recent study determined that only one of three commercial standards of microcystin-RR was microcystin-RR; the others were microcystin-RR variants (Kubwabo et al. 2004). From a strictly analytical viewpoint, results from analyses that did not use certified reference standards should not be regarded as qualitative or quantitative.

SAMPLE PREPARATION

The World Health Organization guideline for microcystin concentration in potable water is 1 µg/L and guidelines for the other cyanotoxins are anticipated to be around the same level. Samples containing cyanotoxins must be extracted and concentrated to be quantified by most current analytical methods and bioassays. Drinking water utilities are interested in three types of cyanotoxin concentration measurements: total, intracellular, and extracellular. From a toxicological perspective, total cyanotoxin concentration reflects the consumer's potential exposure, whereas intracellular and extracellular concentrations affect how readily the cyanotoxins will be absorbed after ingestion, as ingested extracellular cyanotoxins are more readily absorbed than intracellular cyanotoxins From an operational perspective, the total cyanotoxin concentration is required for guideline/regulation, whereas the relative amounts of intracellular and extracellular cyanotoxins are needed to derive effective treatment strategies. This section describes sample preparation methodologies for intracellular, extracellular, and total priority cyanotoxins based on several of the preparation methodologies summarized in Table 3-1.

Several factors influence the efficiency of extracting cyanobacterial toxins from water. These include the hydrophobicity and hydrophilicity of the toxins, as well as the complexity of the sample's organic and inorganic matrix. The priority cyanotoxins listed from most hydrophilic to most hydrophobic are cylindrospermopsin, anatoxin-a, and microcystin RR, YR, LR, and LA. Approximately 95% of microcystins are found

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Table 3-1 Concentration and extraction methodologies for intracellular, extracellular, and total cyanotoxins

Concentration	Extraction	Cleanup	Toxins* MCY LR	MCY RR	MCY LA	MCY YR	CYN	ANA	Reference
Plankton net/Freeze-dried	Supercritical fluid extraction	n/a	X	X					Pyo and Shin 1999
Glass-fiber filter	Methanol/water	n/a	X	X	X		X	X	Westrick 2006
Glass-fiber filter	Sonication/Aqueous method extraction	SPE†	X	X		X			ISO 2005
Strained/Dried cells	Methanol/0.2 M HCl	SPE						X	Rawn et al. 2005
Lyophilized cells	Acetonitrile–water–formic acid						X	X	Dell'Aversano et al. 2004
Freeze-dried	Methanol/0.1%TFA						X		Spoof et al. 2006
Lyophilized cells	Ultrasonication, water, methanol						X		Törönké et al. 2004
	SPE		X	X		X			ISO 2005
	SPE disk			X		X		X	Maizels and Budde 2004
	SPE							X	Powell 1997
	Immunoaffinity chromatography		X	X	X	X			Lawrence and Menard 2001, Aranda-Rodriguez et al. 2003
	Solid-phase microextraction (SPME)							X	Ghassempour et al. 2005
Freeze-dried	Methanol/water	SPE	X	X	X	X			Chorus and Bartram 1999
Lyophilized sample	Various techniques		X	X		X			Fastner et al. 2002
Lyophilized cells	Methanol	SPE					X		Li et al. 2001
	Microwave and boiling		X	X	X	X			Metcalf and Codd 2000

*MCY = microcystin; CYN = cylindrospermopsin; ANA = anatoxin-a.
†SPE = solid-phase extraction.

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intracellularly during a healthy bloom (Chorus and Bartram 1999). When cell growth slows and the population begins to senesce, a larger proportion of intracellular toxin is released into the water. Cylindrospermopsin is naturally released from the cyanobacterial cells; the reported ratio is about 50% intracellular and 50% extracellular during exponential phase, with more toxin released during senescence (Griffiths and Saker 2003). Little is known regarding anatoxin-a distribution. The discussions that follow will review published sample preparation techniques and will guide operators and managers in developing in-house methodologies until standard methods are published by USEPA.

Intracellular Cyanotoxins

Source water monitoring typically employs extraction methods for intracellular cyanotoxins because the methodology is quick and simple. The steps consist of cell concentration, cell lyses, cyanotoxin extraction, and a final sample cleanup to remove particulates prior to analysis. The most common concentration method to isolate cyanobacteria cells from the extracellular toxins is glass fiber filtration (Lawton et al. 1994, Spoof et al. 2003). A less common method is centrifugation. The cyanobacteria cells are lysed by one of five methods: (1) a series of freeze–thaw steps; (2) mechanical homogenization; (3) sonic homogenization; (4) boiling; (5) microwave; and (6) a new, commercially available method that uses detergents to break cell membranes, combined with freezing to lyse cells and release toxin (Abraxis™). The most common method to disrupt the cell is freeze–thaw; however, some sonication methods have been noted as being more effective at cell disruption (Rapala et al. 2002b, Osswald et al. 2007).

The cyanotoxin lysate extraction methodology has been developed mainly to extract one cyanotoxin or one group of cyanotoxins. Microcystins can be extracted with acidified water (Harada et al. 1988), methanol (Lawton et al. 1994), acidified methanol/water mixtures (Nicholson and Burch 2001), and butanol/methanol/water mixtures (Harada et al. 1996). A more recent study performed by Barco and coworkers (2005) suggests that the most efficient method for the extraction of intracellular microcystins is with acidified methanol (about pH 2). Törökné et al. (2004) reported the results of cylindrospermopsin lysate extraction from a six-lab methodological study. They concluded that 5% formic acid for cylindrospermopsin extraction provided the best extraction of cellular material (with the least contaminants). Harada et al. (1989) compared anatoxin-a extraction efficiency on a cultured cyanobacterial strain using four solvents and concluded that 0.5M acetic acid–water had highest recovery. Rapala et al. (1993) evaluated anatoxin-a extraction of freeze-dried algal samples and concluded that water was the most efficient extraction solvent, however recommended using 50% methanol to reduce peak interferences while sacrificing little efficiency.

With several reports of more than one of the priority cyanotoxins being present in one sample, methodology to efficiently extract the priority cyanotoxins is needed. An efficient method must account for the varying hydrophobicity of the priority cyanotoxins. The priority cyanotoxins have been successfully extracted from lysate with sequential extractions consisting of polar 80:20 aqueous methanol followed by a less polar 20:80 aqueous methanol (Westrick 2006). Using a polar/less polar extraction sequence has given recoveries for the priority cyanotoxins from fortified natural samples, cylindrospermopsin, anatoxin-a, microcystin-LR, microcystin-RR, and microcystin-LA of 90%, 92%, 97%, 94%, and 93%, respectively. The extracts can be directly analyzed or concentrated by evaporation under dry nitrogen.

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Extracellular Cyanotoxins

Extracellular cyanotoxin preparation techniques are needed for high-performance liquid chromatography (HPLC)/ultraviolet (UV) and liquid chromatography/mass spectrometry (LC/MS) analytical methods to have limits of detection in the ppb and ppt range. The preparation steps include removing cells from the sample, concentration, and cleanup. Filtration and/or centrifugation of the sample are used to remove the cyanobacteria and other particulates. Freeze-drying, solid-phase extraction, and immunoaffinity chromatography methods concentrate the particulate free sample. The freeze-dried sample is extracted with either water or aqueous methanol as mentioned in the Intracellular section. This concentration method, though time-consuming, gives high recovery of the priority cyanotoxins.

The simplest and most time-efficient methods for concentrating cyanotoxins from particulate free water are solid-phase extraction and immunoaffinity chromatography. The major disadvantage of these methods is that each group of the priority cyanotoxins uses different column media based on the hydrophobicities of the cyanotoxins. (Hudnell 2008, Nicholson et al. 2007). Microcystins are commonly concentrated with reversed-phase octadecyl (C_{18}) silanized silica gel (ODS) cartridges. Several commercially available solid-phase cartridges have been evaluated (Meriluoto et al. 1996). The disadvantage of the solid-phase extraction step is that while microcystin-LR recovery is reported frequently above 85%, other variants such as microcystin-LA or microcystin-LW are reported to have a much lower percent recovery (Lawton et al. 1994, Westrick et al. 2006). Immunoaffinity chromatography methods have also been used to concentrate and clean up microcystin water samples (Lawrence and Menard 2001, Aranda-Rodriguez et al. 2003). Although several publications have demonstrated that the immunoaffinity column methodology is an efficient analytical technique, no commercial columns are available. Anatoxin-a has been concentrated using a weak cation ion-exchange solid-phase extraction (James et al. 1998). Furey et al. (2002) recommended MegaBond C_{18} cartridges with pH adjustment to 9.6. As a large portion of cylindrospermopsin is typically dissolved in water, the use of activated carbon/C18 cartridges is recommended to extract and concentrate this analyte (Norris et al. 2001, Metcalf et al. 2002, Meriluoto and Codd 2005). With the development of a cylindrospermopsin antibody (Abraxis), a potentially powerful concentration procedure would be an immunoaffinity chromatography method with both microcystin and cylindrospermopsin antibodies. The development of a mixed-mode media with both reverse-phase and ion-exchange functionalities for the simultaneous solid-phase extraction of the priority cyanotoxins would greatly simplify cyanotoxin extraction.

Solid-phase extraction is typically used for sample concentration and cleanup, however there are anatoxin-a and cylindrospermopsin sample preparation references that use solid-phase extraction method as only a cleanup step in the literature. In this practice the volume of concentrated sample added to the column is equal to or slightly greater than the load of capacity of the column; all eluants (including the rinse) are collected. Cleanup procedures for anatoxin-a and cylindrospermopsin include using C_{18}, Carbograph™, and HLB Oasis™ solid-phase extraction columns with reported recoveries to be above 85% (Nicholson et al. 2007).

TOTAL CYANOTOXINS

Total cyanotoxin extraction and concentration methodologies include concentration, cell lysing, and extraction. One methodology concentrates the sample by freeze-drying and then extracts the toxins as mentioned in the Intracellular Cyanotoxins section and summarized in Table 3-1 (Chorus and Bartram 1999, Nicholson et al. 2007). The other methodology first lyses the cells by either freeze–thaw, mechanical, or sonic

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homogenization and then concentrates and extracts the cyanotoxins by the techniques mentioned in the Extracellular Cyanotoxins section. The other approach would be to add the intracellular and extracellular concentrations together to give the total concentration.

Summary

This section focused on sample preparation methodologies for the priority cyanotoxins. It is important for drinking water managers to understand the importance of quantifying the three types of cyanotoxin concentrations, intracellular, extracellular, and total, from regulatory and treatment perspectives. Currently, more sample preparation studies have been performed on the microcystin and microcystin variants than cylindrospermopsin and anatoxin-a. The only extraction and concentration techniques for all of the priority toxins would be to freeze-dry the sample and follow with a sequential polar/less-polar extraction. Drinking water managers must understand that certified standards are not commercially available for the priority cyanotoxins and must use this information in selecting a commercial laboratory or developing an in-house analytical methodology. Although microcystins are extremely stable and resistant to degradation under standard environment conditions, extra care must be given to samples containing anatoxin-a and cylindrospermopsin by keeping them cold and in the dark to minimize degradation. Future research would be to investigate one step concentrate and cleanup sample preparation methodology and also the development of internal standards. The potential areas of sample preparation research include multimedia solid-phase extraction and multi-antibody immunoaffinity chromatography. The exploration of semisynthetic isotopic labeled cyanotoxins may lead to internal standards and surrogates for validated analytical methods useful for specialized applications (MS analyses).

SCREENING ASSAYS

The screening assays for cyanotoxins consist of two types: biochemical and enzyme-linked immunosorbent assays (ELISA). These assays are very sensitive but have low selectivity. Assays can usually measure subnanogram analyte quantities, but frequently the binding site will cross-react with other compounds. Typically of the two types of assays, ELISA is more selective than biochemical assays. Biochemical assays are sometimes referred to as toxicity assays as either an inhibition infers that a metabolic pathway is altered or some level of toxicosis results from exposure.

One type of biochemical assay screens classes of compounds based on enzyme inhibition. The amount of inhibitor is inversely proportional to the inhibition of enzymatic reaction. The class of cyclic peptide cyanotoxins, microcystin and nodularin, inhibits protein phosphatase 2a and partially types 1 (Komatsu et al. 2007). Protein phosphatase is an intercellular protein that dephosphorylates phospho-protein-particular serine/threonine phosphatases. Phosphatase inhibition directly impacts glycoysis, as well as energy transfer through nicotinamide adenine dinucleotide phosphate (NADP) and adenosine triphosphate (ATP). These cyanotoxins inhibit protein phosphatase *in vivo* and have been linked to hepatotoxicity (Chorus and Bartram 1999). Furthermore, research suggests a direct relationship between inhibition of protein phosphatase and tumor promotion (Fujiki and Suganuma 1999). The protein phosphatase inhibition assay developed by An and Carmichael (1994) is an extremely sensitive technique. Phosphatase inhibition assays may give an overestimation of toxicity as the LR form is the most toxic of the variants. Often the results from this assay are reported in microcystin-LR toxicity equivalents instead of less potent cogeners present (Nicholson et al. 2007). Further research is needed to evaluate the priority microcystin congeners LA and YR in terms of toxicity.

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Immunoassays use antigen/antibody interactions to identify and quantify chemical contaminants. Antibodies are proteins that are produced by an immune system in response to the presence of a foreign material (antigen) and thereby defend the body by forming an antigen/antibody complex that is nontoxic and can be excreted. Cyanotoxin-selective antibodies have been developed and incorporated into both quantitative and semiquantitative assays to successfully screen for cyanobacterial toxins. These commercially available kits are simple and serve as (near) real-time assays that can be performed in the field or laboratory.

Immunoassays are divided into competitive and noncompetitive type assays. In a *competitive* immunoassay, the antigen in the unknown sample competes with labeled antigen to bind with antibodies. The amount of labeled antigen bound to the antibody site is then measured. In this method, the response is inversely proportional to the concentration of the antigen in the unknown, so the greater the response, the less antigen in the unknown was available to compete with the labeled antigen. In a *noncompetitive* immunoassay, also referred to as the "sandwich assay," antigen in the unknown is bound to the antibody site, then the labeled antibody is bound to the antigen. The amount of labeled antibody on the site is then measured. Unlike the competitive method, the results of the noncompetitive method are directly proportional to the concentration of the antigen, as the labeled antibody will not bind if the antigen is not present in the unknown sample.

As immunoassays detect picograms of antigen, the measurable signal is created by an amplification technique. Commonly, enzyme or fluorescent tags amplify the original signal. The colorimetic or fluorescence response is referred to as either direct or indirect measurable responses. If the response is created by the antigen or antigen derivative, then it is referred to as a direct response. When the measured response is created by a derivatized antibody to make a derivatized antibody/antigen/antibody complex, it is considered to be an indirect response. For example, several of the microcystin ELISA kits are direct competitive assays. This means that the microcystin in the sample and a derivatized microcystin compete to bind the antibodies on the plate. The derivatized antigen will produce a measurable response that is inversely related to the amount of microcystin. Several research groups are investigating new technologies to develop small molecule antigen noncompetitive ELISA tests that will increase sensitivity for detecting these toxins.

Companies producing microcystin ELISA kits use different strategies to develop their antibodies as well as kit designs. Microcystin immunoassays use either polyclonal or monoclonal antibodies and are designed as either direct or indirect competitive assays. The enzyme used in all cyanotoxin commercial kits is horseradish peroxidase (HRP), which oxidizes 3,3', 5, 5'-tetramethylbenzidine (TMB) to produce a deep blue color. However, it is the clear yellow color that is formed after addition of the acidic stop solution that is detected at 450 nm to determine the microcystin levels. Each of the kits addresses cross-reactivity of the microcystins in their instructions. These assays are marketed in microplate, test tube, and strip formats. An overview of each product is summarized in Table 3-2.

The Envirologix™, Abraxis (Adda), Enviroguard™, and Beacon™ kits employ polyclonal antibodies raised against microcystin-LR. Antibodies raised against a specific congener provide great sensitivity and stability in detecting microcystins with the Adda-arginine peptide region (LR, RR, and YR). The Abraxis DM uses a monoclonal antibody raised against the Adda group. This monoclonal antibody was designed to offer the advantage of being able to detect the various microcystin congeners with nearly equal sensitivity. Several studies have examined cross-reactivity, accuracy, and precision of microcystin ELISA kits (Nicholson et al. 2007, McElhiney and Lawton 2005, Metcalf and Codd 2003).

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Table 3-2 Summary of available ELISA microplate assays for the priority cyanotoxins

Microplate Kits	Type of ELISA	Type of Antibody and Target Antigen	Sample Size (µL)	Detection Range (ppb)	Effort, Time to Perform Assay (steps, minutes)	Cost (US dollars)	Cross-Reactivity 50% B/B_0 (ppb)
Section A: Microcystin ELISA							
Envirologix–Microcystin	Direct competitive	Polyclonal – MCY LR	20 50	Source waters, 0.16–2.5; Finished water*, 0.05–0.83	3, 90	$396	
MCY LR							0.50 (100%)
MCY RR							0.92 (54%)
MCY LA							0.81 (62%)
MCY YR							1.42 (35%)
Abraxis DM ELISA	Direct competitive	Monoclonal–Adda	100	0.15–5.0	2, 110	$360	
MCY LR							0.66 (100%)
MCY RR							1.24 (53%)
MCY LA							1.39 (47%)
MCY YR							1.03 (64%)
Abraxis ELISA (Adda) kit	Indirect competitive	Polyclonal–Adda	50	0.15–5.0	3, 150	$400	
MCY LR							0.62 (100%)
MCY RR							0.69 (90%)
MCY LA							0.50 (124%)
MCY YR							0.80 (78%)
Strategic Diagnostics–Enviroguard Microcystin	Direct competitive	Polyclonal–MCY LR	100	0.16–1.60	3, 90	$440	
MCY LR							0.31 (100%)
MCY RR							0.32 (97%)
MCY LA							1.35 (23%)
MCY YR							0.38 (82%)
Beacon	Direct competitive	Polyclonal–MCY LR	50	0.1–2.0	2, 60	$240	
MCY LR							0.48 (100%)
MCY RR							0.55 (87%)
MCY LA							>4.8 (<10%)
MCY YR							1.04 (46%)
Section B: Cylindrospermopsin ELISA							
Beacon	Direct competitive		50	0.1–2.0	2, 90	$300	
Abraxis	Direct competitive	Polyclonal–cylindrospermopsin	50	0.05–2.0	2, 75	$500	

*Separate calibrators are available.

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In the last two years, cylindrospermopsin ELISA kits have become commercially available (Table 3-2). Both kits are indirect competitive assays using the HRP, which oxidizes 3,3',5, 5'-TMB to produce a colorimetric response. Cylindrospermopsin immunoassays are marketed only in the microplate format.

Several groups have attempted to make antibodies to anatoxin-a. To date, these efforts have not been successful. Undoubtedly, this is due to the extremely small size of the toxin relative to the linker complex. Antibodies produced to date cross-react with linker proteins, thus efforts continue to reduce linker size and reactivity.

Although numerous organic and inorganic compounds commonly found in water samples have been tested and do not interfere with the cyanotoxin ELISA and biochemical assays, variability in sample water quality may lead to unreliable results. Positive test results from these assays should be confirmed by a quantitative method such as LC/MS to provide two independent detection methods. Cyanotoxin immunoassays are excellent screening tools for the drinking water industry.

Summary

Biochemical inhibition and ELISAs can be used as screening tools in the drinking water industry. The advantage of these assays over the analytical methods is that samples do not have to be concentrated and results can be finalized within hours. These assays can be used in-house to screen source and finished waters, to quantify toxins for bench and pilot studies, and to evaluate the removal efficiency of a unit process.

ANALYTICAL TECHNIQUES

Microcystin

Microcystin analyses have used (primarily) reverse-phase columns and elution (Siegelman et al. 1984, Chorus and Bartram 1999, Meriluoto and Codd 2005); additionally internal surface reverse-phase (Meriluoto et al. 1990) and anion-exchange neutral-phase columns have provided good separation (Gathercole and Thiel 1987). While C_{18} reverse-phase columns have been used most often in separations (Meriluoto and Codd 2005), other columns such as C_{16} amide columns have been shown to be extremely good for separations (Spoof et al. 2001).

Historically, microcystin analyses used a neutral mobile phase (water) and acetonitrile with ammonium acetate resulting in pH ranging from 8.4 to 10 (Siegelman et al. 1984). Siegelman and colleagues (1984) purified microcystin-LR using this procedure. A major development was the use of acidic mobile phases using trifluoroacetic acid (TFA) (Krogman et al. 1986) in a gradient elution procedure; this method was used to successfully separate five microcystin variants by Wicks and Thiel (1990). More recently, Lawton and colleagues (1994) further refined understanding of the elution order of variants using 0.05% TFA in both the water and acetonitrile mobile-phase components. Arginine-containing variants eluted prior to the uncharged and more hydrophobic species. Elution order was -dimethyl RR, -RR, -YR, -LR, -FR, -LA, -LY, -LW, and -LF.

There are few studies comparing different mobile and stationary phases (Spoof et al. 2001, Rapala et al. 2002a). Spoof and colleagues (2001) reported that better separation was achieved using acidic mobile phases relative to ammonium acetate; however, demethylated forms of LR were not separable using acidified mobile phases but were separated when using ammonium acetate. Formic acid had less UV interference than TFA and yielded better chromatography.

While detection of microcystins via UV light is relatively straightforward (as the conjugated diene in the Adda portion of the molecule has strong absorbance at 238 nm),

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identification of the 80 or so variants of this molecule is not possible. One approach to help in recognizing subgroups is through other absorbance patterns. Variants containing tryptophan have an additional absorbance peak at 222 nm, which is useful for recognition, whereas the flattened UV spectra between 230 and 240 nm is found in tyrosine-containing microcystins (Lawton et al. 1994).

Poon et al. (1993) pioneered the use of electrospray ionization mass spectrometry in cyanotoxin research. Another student of Geoff Codd advanced MS detection by confirming the 135-amu peak associated with the Adda terminus as a means of rapidly screening samples for presence of microcystins (Edwards et al. 1993). Quantitative analyses using electrospray ionization mass spectrometry have been validated using fortified animal tissues (Karlsson et al. 2005). The use of ultra high-pressure columns and smaller particle sizes has increased throughput. When these column developments are coupled with tandem mass spectrometers, it is possible to screen samples within 2.8 minutes per analysis (Meriluoto et al. 2004) if the 135-amu Adda fragment is monitored. A summary of the UV maxima and MS ions of the priority cyanotoxins is presented in Table 3-3.

Harada et al. (1996) validated methodology which suggested that the formation of microcystin fragments such as of 3-methoxy-2-methyl-4-phenylbutyric acid could be used to monitor microcystin abundance (Sano et al. 1992). This method involves sample oxidation using ozonolysis, with detection based on gas chromatography/mass spectrometry (GC/MS) or fluorescence detection after HPLC separation.

Kondo and colleagues (1992, 1995) pioneered the use of frit fast bombardment techniques for microcystin detection. Howard and Boyer (2007) developed laser-assisted time-of-flight assessment of microcystins, demonstrating quantification was possible using these methods.

The microcystin ISO method has been validated on both source and finished drinking water and uses reverse-phase solid-phase extraction to concentrate and clean up the sample and HPLC with UV detection (ISO 2005). In this method, the MS may be used in addition to the UV detector to confirm the presence of microcystins. This method has been confirmed for three of the four priority microcystin variants, LR, RR, and YR. The recovery for microcystin-LR was greater than 85%, while the recoveries for microcystin RR and YR were greater than 70%. The variation in the microcystin recoveries is due to the wide range of hydrophobicity. The detection limit for HPLC/UV is about 1 ng per injection.

Anatoxin-a

Anatoxin-a was the first cyanobacterial toxin to have its structure elucidated (Devlin et al. 1997). The compound is a neurotoxin that is a potent nicotinic agonist.

To date, toxic incidents involving anatoxin-a have been limited to cattle, dogs, and birds (reviewed in Furey et al. 2002); however, the high toxicity at low levels poses a serious health concern for humans and pets. While anatoxin-a is on the USEPA priority toxin list, homoanatoxin, which is less stable than anatoxin, should also be measured (Furey et al. 2002). In all cases, C18 reverse columns have been recommended for anatoxin analyses. Several different detectors have been used after successful separation by HPLC, including photodiode array (PDA), fluorescence, and mass spectrometry. Early techniques utilized photodiode array detection of anatoxin-a (Harada et al. 1989); difficulties with the method include the lack of a strong absorbance for the anatoxin-a molecule. For this reason few researchers have continued use of this PDA method.

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Table 3-3 Physical properties of priority microcystins and other cyanotoxins

Variant		Absorbance	Mass (amu)	Fragmentation Ions
Microcystin	-LR	238	995	570, 553,285,163,135
	-RR	238	1,037 (519)	519, 440, 277 (281?), 185, 147, 135
	-YR	238	1,044	135
	-LA	238	909	874, 163, 135
	-LF	224	985	583, 491, 375, 268, 249, 163, 135
	-LW	238	1,024	1,008, 874, 583, 517, 446, 375, 288, 163
Cylindrospermopsin		262	415	299, 176, 141, 100
Anatoxin		240	165	
Variant		Fluorescence Excitation/Emission	Mass (amu)	Fragmentation Pattern
Anatoxin-a		470/530	165	165 (328 w/NDB) 162, 145, 107, 95
Homoanatoxin-a		470/530 (225 nm UV absorbance maximum)	179 (342 w/ NDB)	162, 145, 1

Several methods have been used for fluorescence derivatization of anatoxin. James et al. (1997) showed that 4-nitro-2,1,3-benzoxadiazole (NBDF) precolumn derivatization methods yielded over 85% recovery. More recently, Rawn et al. (2007) modified the James et al. method via sample pretreatment with mercaptoethanol and *o*-phthaldialdehyde to remove primary amines prior to addition of the NBDF fluorescent dye, resulting in lower detection limits. This latter method is the current Association of Official Analytical Chemists (AOAC) standard method.

Detection of anatoxin-a by single quadrupole MS can be difficult in light of the similar mass of phenylalanine. As a confirmatory assessment, MS would not be sufficient. While the fragmentation products can be monitored (e.g., 41, 63, and 165 amu), the ratio of these fragments must be measured by MS/MS or MALDI-TOF MS techniques. Takino et al. (1999) developed in-line fluorescence derivatization coupled with MS/MS, an analysis that has not been rigorously assessed. In past years, phenylalanine has been misidentified as anatoxin-a using mass spectrometry (Furey et al. 2005). This occurred because anatoxin-a and phenylalanine have molecular weights of 165.07898 amu and 166.0868 amu, respectively. Furey and coworkers (2005) suggested several approaches to prevent misidentification of anatoxin-a, including derivatization and fluorimetric LC, methylation using diazomethane and LC/MS, multiple tandem MS using a quadrupole ion-trap, and hybrid quadrupole time of flight.

Several GC/MS methods have been devised for identification of anatoxin. Edwards et al. (1992) demonstrated equivalent recovery using GC/MS and LC/MS methods. Sensitivity for most of these methods was similar to electron capture detection (Osswald et al. 2007). Araoz and colleagues (2005) used ligand binding to isolate anatoxin, followed by GC/MS detection.

Cylindrospermopsin

Cylindrospermopsin exhibits a strong absorbance at 262 nm, which is useful for detection. Harada et al. (1994) pioneered HPLC analyses, using an isocratic 5% MeOH mobile phase. Hawkins and colleagues (1997) used a linear gradient 0%–10% MeOH. Törökné et al. (2004) reported on the results of a six-lab methodological comparison of cylindrospermopsin measurement and concluded that isocratic conditions of 5% MeOH

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with 0.1% TFA was the best mobile phase. Typically C18 columns were used in these analyses. Meriluoto and Codd (2005) followed these recommendations in their methodological review and provided methodology for cylindrospermopsin measurement. The parent ion of 415 amu coupled with the 262-nm absorbance is easy to use for identification, particularly if SIM mode is used to track fragments.

Multiple Cyanotoxins in a Single Method

As stated earlier, several cyanotoxins may be present in one cyanobacterial bloom, and several genera of cyanobacteria have the capability to produce more than one toxin. For example, *Planktothrix* and *Anabaena* can simultaneously produce both anatoxin-a and microcystins, while *Aphanizomenon* can produce both cylindrospermopsin and anatoxin-a. Simultaneous determination of the cyanotoxins is of the utmost importance because it conserves supplies, instrumentation use, and time. Only a handful of scientists (Oehrle and Westrick 2003, Maizels and Budde 2004, Snyder et al. 2004, Oehrle et al. 2010, Nicholson et al. 2007) have published methodologies that further simultaneous measures.

Oehrle and Westrick (2003) developed a single-run analytical method that targeted microcystins, anatoxin-a, and nodularin using LC/MS. This technique utilized a reverse-phase column (YMC Pro C18 column, Waters Corp.) and a single quadrupole mass spectrometer. Anatoxin-a, nodularin, and microcystins (RR, YR, and LR) were easily detected at a level of 2.5 µg/L without any sample preparation beyond filtering. This method was further developed to include cylindrospermopsin and other variants of microcystins (Oehrle et al. 2010). The initial method was developed on an HPLC/PDA and then converted to LC/MS. Both HPLC/PDA and LC/MS used a reverse-phase column with polar compound separation. The HPLC/PDA had detection limits of 0.25 µg/L for each of the toxins with a thousandfold preconcentration, and the LC/MS had detection limits of 50 ng/L for each of the toxins with a thousandfold preconcentration. Snyder and coworkers (2004) also presented an LC/MS/MS method with six of the priority cyanotoxins: cylindrospermopsin, anatoxin-a, nodularin, and microcystin LR, RR, and YR. The toxins were separated by reverse-phase chromatography and detected by tandem mass spectrometric detection. Electrospray ionization in both negative and positive mode was employed. The reporting limit of 0.50 µg/L was established for the six cyanobacteria toxins. Maizels and Budde (2004) developed a multi-cyanotoxin method to measure anatoxin-a and microcystin LR, RR, and YR. The toxins were separated by reverse-phase liquid chromatography, and limit of detection was about 50 ng with a benchtop time-of-flight (TOF) mass spectrometer. These methods need to be developed further to measure the six priority cyanotoxins: microcystin LR, RR, YR, and LA, cylindrospermopsin, and anatoxin-a.

Nicholson and coworkers (2007) developed a single-run LC/MS/MS analytical method to determine levels of cylindrospermopsin, anatoxin-a, and four saxitoxins. The following detection limits without preconcentration for cylindrospermopsin, anatoxin-a, and saxiton have been achieved: 0.1 µg/L, 1 µg/L, and 50 µg/L, respectively. The method used hydrophilic liquid chromatography instead of the standard reverse-phase chromatography.

Summary

The USEPA has identified the priority cyanotoxins to be microcystin LR, RR, LA, and YR, anatoxin-a, and cylindrospermopsin. These cyanotoxins have moved from the Contaminant Candidate List (CCL) to the 2011 Unregulated Contaminant Monitoring Rule List. Although the USEPA has not published a standard method for the priority cyanotoxins, three methods have gained international approval, two for use in microcystin

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analyses (Lawton et al. 1994, ISO 2005) and one for use in anatoxin-a analysis (ISO 2005, Rawn et al. 2005). Both microcystin analyses are HPLC/PDA methods using C18 columns, acetonitrile:water:TFA as mobile phase, and run times around 55 minutes. These methods can use MS for secondary confirmation; however, the TFA concentrations typically are reduced from 0.05% to 0.01% to reduce inlet damage during electrospray detection. The validated anatoxin-a analysis method is a derivatization using fluorimetric LC. No cylindrospermopsin method or no multiple cyanotoxin method has gained international approval.

QUALITATIVE AND QUANTITATIVE METHODS FOR TOXIC CYANOBACTERIA

Microscopic enumeration and identification has been used widely to identify the composition and density of algae blooms. Several health and recreational management guidelines have been based on these microscopic analyses of specific taxa known to produce toxins (Chorus and Bartram 1999, Yoo et al. 1995). Although detailed identification and enumeration can take several hours for a sample, microscopically screening samples to determine the water quality and bloom status can be performed within 20 minutes by a trained phycologist. The major disadvantages of using microscopic identification to predict toxic cyanobacteria blooms include (1) the need for a trained phycologist, (2) intercalibration with other laboratories for consistency, (3) only species known to be problematic are identified (so species not known to be capable of toxin synthesis are ignored), (4) the algae responsible for toxin formation may not be in the same location as the toxin (grazing, currents, bloom senescence), and (5) toxin-producing cyanobacteria cells look the same as nontoxin-producing cyanobacteria cells. The conservative approach is to assume all genera that are known to potentially produce toxins are toxin-producing, which may greatly overestimate the risk from the cyanobacterial bloom.

One method for separating toxic from nontoxic strains is through DNA analyses, as DNA technologies are rapid, reliable, and accurate. This section discusses the application and the advancements, advantages, and disadvantages of conventional polymerase chain reaction (PCR), quantitative real-time polymerase chain reaction (QRT-PCR), and microarray methods.

Since the difference between nontoxin- and toxin-producing strains of cyanobacteria can not be identified microscopically, scientists have begun scanning the genome of cyanobacteria to help determine the potential risk of toxic cyanobacterial blooms. The gene clusters for microcystin and nodularin production are known and have been fully sequenced from *Microcystis* (Nishizawa et al. 1999, 2000; Tillett et al. 2001; Kaneko et al. 2007), *Planktothrix* (Christiansen et al. 2003), *Anabaena* (Rouhiainen et al. 2004), and *Nodularia* (Moffitt and Neilan 2001). For example, the gene cluster responsible for the biosynthesis of the microcystin in *Microcystis aeruginosa* has two operons, comprises 10 open reading frames, and is transcribed in two groups in opposite directions. Before molecular methods analysis can be developed with high certainty, gene knockout experiments must be used to verify the genes responsible for toxin synthesis and assembly. Gene knockout studies have confirmed the microcystins are produced nonribosomally by the multienzyme complex consisting of polyketide synthases, peptide synthetases, and tailoring enzymes (Dittmann et al. 1997). The biosynthesis of anatoxin-a and cylindrospermopsin are being investigated; however, they remain to be elucidated and verified by gene knockout experiments (Fergusson and Saint 2003). Currently, the polyketide synthase and peptide synthetase gene clusters are being used to detect potential cylindrospermopsin producers; however, knockout studies have not been performed to determine the relevance of these clusters to the

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biosynthesis of cylindrospermopsin. The uses of gene sequences are much more conservative than previous methods using Nif gene presence. These developments provide the potential to detect toxic cyanobacteria by DNA methodologies and can serve as a source water management tool.

Conventional PCR is a fast and cheap method for the detection of potentially toxic strains of cyanobacteria. Like the toxin analyses, the most time-consuming part of the analysis is collecting and preparing the sample. Sample preparation includes DNA isolation, purification, and amplification. Since microcystin is found worldwide and the 55-kb gene clusters have been verified by gene knockout experiments, most PCR studies focused initially on detecting toxic *Microcystis* strains and only recently progressed to include the other microcystin producers summarized in Table 3-4. McyA and mcyE are two of the most studied regions of the gene. McyE constructs the toxic Adda and adjoining glutamate, which is a conserved region for both microcystin and nodularin. It has successfully identified nodularin- and microcystin-producing cyanobacteria. Researchers have suggested to use mcyE as a surrogate for hepatotoxicity, and mcyA or B as a surrogate for microcystin toxicity. Research suggests that rarely is the microcystin gene cluster present without toxin being synthesized (Tillett et al. 2001, Kaebernick et al. 2001, Nonneman and Zimba 2002, Mikalsen et al. 2003). Recently Kurmayer et al. (2004) showed that a rather high frequency of the *Planktothrix* strains/filaments contained a portion of these genes but were not producing microcystins. If this is correct, the assumption of constitutive toxin synthesis must be investigated. Further research must evaluate portions of the microcystin molecule that are synthesized from these altered pathways that have biological activity (Gulledge et al. 2003).

Real-time polymerase chain reaction (RT-PCR), also called quantitative real-time polymerase chain reaction (QRT-PCR) is a high-throughput laboratory technique based on PCR. Fluorescent dyes are used to quantify the copy DNA strands present, thereby allowing estimates of sample copy numbers present. More than one dye can be used per sample, allowing identification of multiple target DNA molecules per sample. This practice is referred to as multiplex PCR; a multiplex reaction has been developed to detect *Microcystis*-specific 16S rDNA and microcystin toxin synthetase genes mcyB and mcyD (Nonneman and Zimba 2002, Rinta-Kanto et al. 2005). RT-PCR can also be combined with reverse transcription PCR to quantify low-abundance messenger RNA (mRNA), enabling the quantification of relative gene expression. The quantification of a relative cyanotoxin gene expression may correlate to cyanotoxin concentration. The measurement mRNA would also give scientists a tool for investigating the mechanism that controls toxin production. Two commercially available methodologies, SyberGreen and TaqMan, have been used in developing RT-PCR microcystin and nodularin gene probes. These studies used RT-PCR to monitor lakes for potential toxin-producing cyanobacteria and are summarized in Section B of Table 3-4.

Microarray technology is a new high-throughput laboratory technique based on the Southern Blot methodology that has not been widely used in environmental analyses. The microarray oonsists of small dots of DNA embedded in a solid matrix. Each dot represents a target sequence of DNA, such as gene. Most frequently, mRNA is isolated from cells, cDNA containing fluorophore-labelled nucleotides is constructed using reverse-transcriptase PCR, and the fluorescent cDNA binds to the complementary DNA in the microarray dot, producing a fluorescent signal. In theory, DNA microarray probes could be designed to identify nontoxic and toxic cyanobacteria as has been done with toxic marine diatom species (Schèolin et al. 2004). Several research groups are investigating this technology to use as a tool in source water management of hazardous cyanobacterial blooms.

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Table 3-4 Published DNA methodologies for microcystin-producing cyanobacteria

Target Organism	Gene	Reference
Section A: Conventional PCR analyses		
Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya, Microcystis, Nodularia, Nostoc, Planktothrix, Plectonema, Pseudanabaena, Synechococcus	General primers for peptide and microcystin synthetases	Neilan et al. 1999
Microcystis	mcyA	Tillett et al. 2001
Microcystis	mcyB	Pan et al. 2002
Microcystis	mcyB	Nonneman and Zimba 2002
Microcystis	mcyB	Kurmayer et al. 2002, Kurmayer and Kutzenberger 2003
Anabaena, Microcystis	mcyE	Vaitomaa et al. 2003
Anabaena, Microcystis, Planktothrix	mcyA, mcyB	Hisbergues et al. 2003
Microcystis	mcyA, mcyB	Via-Ordorika et al. 2004
Anabaena, Microcystis, Planktothrix, Nostoc, Nodularia	mcyE, mcyD	Rantala et al. 2004
Planktothrix	mcyA	Kurmayer et al. 2004
Planktothrix	mcyA, mcyB, and mcy C	Kurmayer et al. 2005
Microcystis	mcyA, mcyB, mcyC, mcyD, mcyE and mcyG	Ouahid et al. 2005
Planktothrix	mcyT, TD, A, EG, B, E CJ, HA	Mbedi et al. 2005
Microcystis	mcyB and mcyD	Hotto et al. 2005
Microcystis, Planktothrix	mcyA	Rinta-Kanto and Wilhelm 2006
Microcystis	mcyA	Yoshida et al. 2007
Section B: RT-PCR analyses		
Microcystis	mcyA /TaqMan	Foulds et al. 2002
Microcystis	mcyB/TaqMan	Kurmayer and Kutzenberger 2003
Anabaena, Microcystis	mcyE/SyberGreen	Vaitomaa et al. 2003
Microcystis	mcyD/ TaqMan	Rinta-Kanto et al. 2005
Microcystis	mcyB/TaqMan	Schober et al. 2007

Summary

DNA technologies have been implemented into clinical and forensic laboratories as reliable diagnostic tools. A careful transition from research to application must occur when dealing with PCR methodology. Two typical PCR methodology mistakes that occur when going from research to application scenarios include (1) the primers are

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always designed and tested only with a subset of strains and thus may not be reliable for environmental samples containing new strain/species, and (2) environmental samples may contain inhibitors that will lead to false negative results or reduce efficiency of DNA cleanup. The transition from research to application must be carefully implemented and evaluated to determine if it is a good tool to manage source water hazardous cyanobacteria blooms.

Currently, the microcystin gene cluster is the only verified cyanobacteria toxin gene cluster that has been verified by gene knockout. Although much is known about the microcystin gene cluster, the mechanism that triggers the expression of the toxin still remains unknown. The current DNA technologies only detect the presence of the gene, not whether the gene is being expressed. These methods are only able to identify potential toxin producers, making the DNA methods a better surrogate for toxic cyanobacteria than microscopic identification; however, it still may produce an artificially high risk factor. Further investigations with these DNA technologies will allow scientists to study toxic cyanobacteria populations *in situ* and the effects of environmental factors on the occurrence and proliferation of toxic cyanobacteria and metabolic regulations of toxin production. Future investigations should include all toxin producers and primers and probes to detect producers of anatoxin-a and cylindrospermopsin.

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REFERENCES

An, J., and W.W. Carmichael. 1994. Use of a colorimetric protein phosphatase inhibition assay and enzyme linked immunosorbent assay for the study of microcystins and nodularins. *Toxicon*, 32(12):1495–1507.

Aranda-Rodriguez, R., C. Kubwabo, and F.M. Benoit. 2003. Extraction of 15 microcystins and nodularin using immunoaffinity columns. *Toxicon*, 42(6):587–599.

Araoz, R., H-O. Nghiem, R. Rippka, N. Palibroda, N.T.d. Marsac, and M. Herdman. 2005. Neurotoxins in axenic cyanobacteria: coexistence of anatoxin-a and homoanatoxin-a determined by ligand-binding assay and GC/MS. *Microbiology*, 151:1263–1273.

Barco, M., L.A. Lawton, J. Rivera, and J. Caixach. 2005. Optimization of intracellular microcystin extraction for their subsequent analysis by high-performance liquid chromatography. *Jou. Chromatogr.*, 1074:23–30.

Chorus, I., and J.F. Bartram. 1999. *Toxic cyanobacteria in water: A guide to their public health consequences, monitoring, and management*. London: E&FN Spon.

Christiansen, G., J. Fastner, M. Erhard, T. Borner, and E. Dittmann. 2003. Microcystin biosynthesis in *planktothrix*: Genes, evolution, and manipulation. *Jour. Bacteriol.*, 185(2):564–572.

Dell'Aversano, C., G.K. Eaglesham, and M.A. Quilliam. 2004. Analysis of cyanobacterial toxins by hydrophilic interaction liquid chromatography-mass spectrometry. *Jour. Chromatogr.*, 1028(1):155–164.

Devlin, J.P., O.E. Edwards, P.R. Gorham, N.R. Hunter, R.K. Pike, and B. Stavric. 1997. Anatoxin-a, A toxic alkaloid from *Anabaena flos-aquae* NRC-44h. *Can. Jour. Chem.*, 55:1367–1371.

Dittmann, E., B.A. Neilan, M. Erhard, H. von Dèohren, and T. Bèorner. 1997. Insertional mutagenesis of a peptide synthetase gene that is responsible for hepatotoxin production in the cyanobacterium *Microcystis aeruginosa* PCC 7806. *Molec. Microbiol.*, 26(4):779–787.

Ecobichon, D.J. 1997. *The basis of toxicity testing*. Boca Raton, Fla.: CRC Press. p. 220.

Edwards, C., L.A. Lawton, K.A. Beattie, G.A. Codd, S. Pleasance, and G.J. Dear. 1992. Analysis of microcystins from cyanobacteria by liquid chromatography with mass spectrometry using atmospheric-pressure ionization. *Rapid Comm. Mass Spectrom.*, 7:714–721.

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Edwards, C., L.A. Lawton, K.A. Beattie, G.A. Codd, S. Pleasance, and G.J. Dear. 1993. Analysis of microcystins from cyanobacteria by liquid chromatography with mass spectrometry using atmospheric-pressure ionization. *Rapid Comm. Mass Spectrom.*, 7:714–721.

Fastner, J., G.A. Codd, J.S. Metcalf, P. Woitke, C. Wiedner, and H. Utkilen. 2002. An international intercomparison exercise for the determination of purified microcystin-LR and microcystins in cyanobacterial field material. *Anal. Bioanal. Chem.*, 374(3):437–444.

Fergusson, K.M., and C.P. Saint. 2003. Multiplex PCR assay for *Cylindrospermopsis raciborskii* and cylindrospermopsin-producing cyanobacteria. *Environ. Toxicol.*, 18(2):120–125.

Foulds, I., A. Granacki, C. Xiao, U. Krull, A. Castle, and P. Horgen. 2002. Quantification of microcystin-producing cyanobacteria and *E. coli* in water by 5'-nuclease PCR. *Jour. Appl. Microbiol.*, 93(5):825–834.

Fujiki, H., and M. Suganuma. 1999. Unique features of the okadaic acid activity class of tumor promoters. *Jour. Cancer Res. Clin. Oncol.*, 125(3-4):150–155.

Furey, A., J. Crowley, A.N. Shuilleabhain, O.M. Skulberg, and K.J. James. 2002. The first identification of the rare cyanobacterial toxin, homoanatoxin-a, in Ireland. *Toxicon*, 41(3):297–303.

Furey, A., J. Crowley, B. Hamilton, M. Lehane, and K.J. James. 2005. Strategies to avoid the mis-identification of anatoxin-a using mass spectrometry in the forensic investigation of acute neurotoxic poisoning. *Jour. Chromatogr. A*, 1082(1):91–97.

Gathercole, P.S., and P.G. Thiel. 1987. Liquid chromatographic determination of cyanoginosins, toxins produced by the cyanobacterium *Micocystis aeruginosa*. *Jour. Chromatogr.*, 408:435–440.

Ghassempour, A., N.M. Najafi, A. Mehdinia, S.S.H. Davarani, M. Fallahi, and M. Nakhshab. 2005. Analysis of anatoxin-a using polyaniline as a sorbent in solid-phase microextraction coupled to gas chromatography–mass spectrometry. *Jour. Chromatogr.*, 1078:120–127.

Griffiths, D.J., and M.L. Saker. 2003. The Palm Island mystery disease 20 years on: A review of research on the cyanotoxin cylindrospermopsin. *Environ. Toxicol.*, 18(2):78–93.

Gulledge, B.M., J.B. Aggen, H. Eng, K. Sweimeh, and A.R. Chamberlin. 2003. Microcystin analogues comprised only of Adda and a single additional amino acid retain moderate activity as PP1/PP2A inhibitors. *Bioorg. Med. Chem. Lett*, 13(17):2907–2911.

Harada, K., K. Matsuura, M. Suzuki, H. Oka, M.F. Watanabe, S. Oishi, A.M. Dahlem, V.R. Beasley, and W.W. Carmichael. 1988. Analysis and purification of toxic peptides from cyanobacteria by reversed-phase high-performance liquid chromatography. *Jour. Chromatogr.*, 448(2):275–283.

Harada, L., K. Harada, Y. Kimura, K. Ogawa, M. Suzuki, A. Dahlem, V. Beasley, and W. Carmichael. 1989. A new procedure for the analysis and purification of naturally occuring anatoxin-a from the blue-green alga *Anabaena flos-aquae*. *Toxicon*, 7:1289–1296.

Harada, K.I., I. Ohtani, K. Iwamoto, M.F. Suzuki, M. Watanabe, K. Watanabe, and K. Terao. 1994. Isolation of cylindrospermopsin from a cyanobacterium *Umezakia natans* and its screening method. *Toxicon*, 32:73–84.

Harada, K., H. Murata, Z. Qiang, M. Suzuki, and F. Kondo. 1996. Mass spectrometric screening method for microcystins in cyanobacteria. *Toxicon: Off. Jour. Int. So. Toxinol.*, 34(6):701–710.

Hawkins, P., N.R. Chandrasena, G. Jones, A.R. Humpage, and I. Falconer. 1997. Isolation and toxicity of *Cylindrospermopsis raciborskii* from an ornamental lake. *Toxicon*, 35:341–346.

Hisbergues, M., G. Christiansen, L. Rouhiainen, K. Sivonen, and T. Borner. 2003. PCR-based identification of microcystin-producing genotypes of different cyanobacterial genera. *Arch. Microbiol.*, 180(6):402–410.

Hotto, A., M. Satchwell, and G. Boyer. 2005. Seasonal production and molecular characterization of microcystins in Oneida Lake, New York, USA. *Environ. Toxicol.*, 20(3):243–248.

Howard, K.L., and G.L. Boyer. 2007. Adduct simplification in the analysis of cyanobacterial toxins by matrix-assisted laser desorption/ionization mass spectrometry. *Rapid Comm. Mass Spectrom.*, 21:699–706.

Hudnell, H.K., ed. 2008. Cyanobacterial harmful algal blooms: State of the science and research needs. In *Advances in experimental medicine and biology*, Vol. 619. New York: Springer.

International Organization for Standardization (ISO). 2005. ISO 20179: *Water quality—determination of microcystins—Method using solid phase extraction (SPE) and high performance liquid chromatography (HPLC) with ultraviolet (UV) detection*. Geneva, Switzerland: ISO.

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James, K.J., I.R. Sherlock, and M.A. Stack. 1997. Anatoxin-a in Irish freshwater and cyanobacteria, determined using a new fluorimetric liquid chromatographic method. *Toxicon*, 35(6):963.

James, K.J., A. Furey, I.R. Sherlock, M.A. Stack, M. Twohig, F.B. Caudwell, and O.M. Skulberg. 1998. Sensitive determination of anatoxin-a, homoanatoxin-a and their degradation products by liquid chromatography with fluorimetric detection. *Jour. Chromatogr. A*, 798(1-2):147–157.

Kaebernick, M., T. Rohrlack, K. Christoffersen, and B.A. Neilan. 2001. A spontaneous mutant of microcystin biosynthesis: genetic characterization and effect on *Daphnia*. *Environ. Microbiol.*, 3(11):669–679.

Kaneko, T., N. Nakajima, S. Okamoto, I. Suzuki, Y. Tanabe, M. Tamaoki, Y. Nakamura, F. Kasai, A. Watanabe, K. Kawashima, Y. Kishida, A. Ono, Y. Shimizu, C. Takahashi, C. Minami, T. Fujishiro, M. Kohara, M. Katoh, N. Nakazaki, S. Nakayama, M. Yamada, S. Tabata, and M.M. Watanabe. 2007. Complete genomic structure of the bloom-forming toxic cyanobacterium *Microcystis aeruginosa* NIES-843. *DNA Res.*, 14(6):247–256.

Karlsson, K.M., L.E.M. Spoof, and J.A.O. Meriluoto. 2005. Quantitative LC-ESI-MS analyses of microcystins and nodularin-R in animal tissue: Matrix effects and method validation. *Environ. Toxicol.*, 20(3):381–389.

Komatsu, M., T. Furukawa, R. Ikeda, S. Takumi, Q. Nong, K. Aoyama, S.-I. Akiyama, D. Keppler, and T. Takeuchi. 2007. Involvement of mitogen-activated protein kinase signaling pathways in microcystin-LR-induced apoptosis after its selective uptake mediated by OATP1B1 and OATP1B3. *Toxicol. Sci.*, 97(2):407–416.

Kondo, F., Y. Ikai, H. Oka, M. Okumura, N. Ishikawa, K.-i. Harada, K. Matsuura, H. Murata, and M. Suzuki. 1992. Formation, characterization, and toxicity of the glutathione and cysteine conjugates of toxic heptapeptide microcystins. *Chem. Res. Toxicol.*, 5(5):591–596.

Kondo, F., Y. Ikai, H. Oka, H. Matsumoto, S. Yamada, N. Ishikawa, K. Tsuji, K. Harada, T. Shimada, M. Oshikata, and M. Suzuki. 1995. Reliable and sensitive method for determination of microcystins in complicated matrices by frit-fast atom bombardment liquid chromatography/mass spectrometry. *Nat. Toxins*, 3:41–49.

Krogman, D.W., R. Butalla, and J. Sprinkle. 1986. Blooms of cyanobacteria on the Potomac River. *Plant Physiol.*, 80:667–671.

Kubwabo, C., N. Vais, and F.M. Benoit. 2004. Identification of microcystin-RR and [Dha7] microcystin-RR in commercial standards by electrospray ionization mass spectrometry. *Jour. AOAC Int.*, 87(4):1028–1031.

Kurmayer, R., and T. Kutzenberger. 2003. Application of real-time PCR for quantification of microcystin genotypes in a population of toxic cyanobacterium *Microcystis* sp. *Appl. Environ. Microbiol.*, 69:6723–6730.

Kurmayer, R., E. Dittmann, J. Fastner, and I. Chorus. 2002. Diversity of microcystin genes within a population of the toxic cyanobacterium *Microcystis* spp. in Lake Wannsee (Berlin, Germany). *Microb. Ecol.*, 43:107–118.

Kurmayer, R., G. Christiansen, J. Fastner, and T. Borner. 2004. Abundance of active and inactive microcystin genotypes in populations of the toxic cyanobacterium *Planktothrix* spp. *Environ. Microbiol.*, 6:1525–1533.

Kurmayer, R., G. Christiansen, M. Gumpenberger, and J. Fastner. 2005. Biodiversity and evolution: Genetic identification of microcystin ecotypes in toxic cyanobacteria of the genus *Planktothrix*. *Microbiology*, 151(5):1525–1533.

Lawrence, J.F., and C. Menard. 2001. Determination of microcystins in blue-green algae, fish and water using liquid chromatography with ultraviolet detection after sample clean-up employing immunoaffinity chromatography. *Jour. Chromatogr.*, 922(1):111–117.

Lawton, L.A., C. Edwards, and G.A. Codd. 1994. Extraction and high-performance liquid chromatographic method for the determination of microcystins in raw and treated waters. *Analyst*, 119(7):1525–1530.

Li, R., W.W. Carmichael, S. Brittain, G.K. Eaglesham, G.R. Shaw, A. Mahakhant, N. Noparatnaraporn, W. Yongmanitchai, K. Kaya, and M.M. Watanabe. 2001. Isolation and identification of the cyanotoxin cylindrospermopsin and deoxy-cylindrospermopsin from a Thailand strain of *Cylindrospermopsis raciborskii* (cyanobacteria). *Toxicon*, 39(7):973–980.

Maizels, M., and W.L. Budde. 2004. A LC/MS method for the determination of cyanobacteria toxins in water. *Anal. Chem.*, 76(5):1342–1351.

Mbedi, S., M. Welker, J. Fastner, and C. Wiedner. 2005. Variability of the microcystin synthetase gene cluster in the genus *Planktothrix* (oscillatoriales, cyanobacteria). *FEMS Microbiol. Lett.*, 245(2):299–306.

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Meriluoto, J.A.O., and G.A. Codd. 2005. *Toxic: Cyanobacterial monitoring and cyanotoxin analysis.* Turku, Finland: Åbo Akademi University Press.

Meriluoto, J.A.O., J.E. Eriksson, K. Harada, A.M. Dahlem, K. Sivonen, and W.W. Carmichael. 1990. Internal surface reversed-phase high performance liquid chromatographic separation of the cyanobacterial peptide toxins microcystin-LA, -LR, -YR, -RR, and nodularin. *Jour. Chromatogr.*, 509:390–395.

McElhiney, J., and L.A. Lawton. 2005. Detection of the cyanobacterial hepatotoxins microcystins. *Toxicol. Appl. Pharmacol.*, 203(3):219–230.

Meriluoto, J., A.S. Harmala-Brasken, J. Eriksson, D. Toivola, and T. Lindholm. 1996. Choosing analytical strategy for microcystins. *Phycologia*, 35(6 Suppl.): 125–132.

Meriluoto, J., K. Karlsson, and L. Spoof. 2004. High-throughput screening of ten microcystins and nodularins, cyanobacterial peptide hepatotoxins, by reversed-phase liquid chromatography–electrospray ionization mass spectrometry. *Chromatographia*, 59:291–298.

Metcalf, J.S., and G.A. Codd. 2000. Microwave oven and boiling waterbath extraction of hepatotoxins from cyanobacterial cells. *FEMS Microbiol. Lett.*, 184(2):241–246.

Metcalf, J.S., and G.A. Codd. 2003. Analysis of cyanobacterial toxins by immunological methods. *Chem. Res. Toxicol.*, 16(2):103–112.

Metcalf, J.S., K.A. Beattie, M.L. Saker, and G.A. Codd. 2002. Effects of organic solvents on the high performance liquid chromatographic analysis of the cyanobacterial toxin cylindrospermopsin and its recovery from environmental eutrophic waters by solid phase extraction. *FEMS Microbiol. Lett.*, 216(2):159–164.

Mikalsen, B., G. Boison, O.M. Skulberg, J. Fastner, W. Davies, T.M. Gabrielsen, K. Rudi, and K.S. Jakobsen. 2003. Natural variation in the microcystin synthetase operon mcyABC and impact on microcystin production in *microcystis* strains. *Jour. Bacteriol.*, 185(9):2774–2785.

Moffitt, M.C., and B.A. Neilan. 2001. On the presence of peptide synthetase and polyketide synthase genes in the cyanobacterial genus *Nodularia*. *FEMS Microbiol. Lett.*, 196(2):207–214.

Neilan, B.A., E. Dittmann, L. Rouhiainen, R.A. Bass, V. Schaub, K. Sivonen, and T. Borner. 1999. Nonribosomal peptide synthesis and toxigenicity of cyanobacteria. *Jour. Bacteriol.*, 181(13):4089–4097.

Nicholson, B.C., and M.D. Burch. 2001. *Evaluation of analytical methods for detection and quantification of cyanotoxins in relation to Australian drinking water guidelines.* Canberra, Australia: National Health & Medical Research Council, Water Services Association of Australia, CRC for Water Quality and Treatment.

Nicholson, B., J. Papageorgiou, A. Humpage, D. Steffensen, P. Monis, T. Linke, S. Fanok, G. Shaw, G. Eaglesham, B. Davis, W. Wickramasinghe, I. Stewart, W. Carmichael, and J. Servaites. 2007. *Determination and significance of emerging algal toxins (cyanotoxins).* Denver, Colo.: Awwa Research Foundation.

Nishizawa, T., M. Asayama, K. Fujii, K. Harada, and M. Shirai. 1999. Genetic analysis of the peptide synthetase genes for a cyclic heptapeptide microcystin in *Microcystis* spp. *Jour. Biochem.*, 126(3):520–529.

Nishizawa, T., A. Ueda, M. Asayama, K. Fujii, K. Harada, K. Ochi, and M. Shirai. 2000. Polyketide synthase gene coupled to the peptide synthetase module involved in the biosynthesis of the cyclic heptapeptide microcystin. *Jour. Biochem.*, 127(5):779–789.

Nonneman, D., and P.V. Zimba. 2002. A PCR-based test to assess the potential for microcystin occurrence in channel catfish production ponds. *Jour. Phycol.*, 38(1):230–233.

Norris, R.L., G.K. Eaglesham, G.R. Shaw, P. Senogles, R.K. Chiswell, M.J. Smith, B.C. Davis, A.A. Seawright, and M.R. Moore. 2001. Extraction and purification of the zwitterions cylindrospermopsin and deoxycylindrospermopsin from *Cylindrospermopsis raciborskii*. *Environ. Toxicol.*, 16(5):391–396.

Oehrle, S.A., and J. Westrick. 2003. Analysis of various cyanobacterial toxins by LC-MS. *LCGC N. Am.*, 21(7):634–640.

Oehrle, S.A., B.J. Southwell, and J.A. Westrick. 2010. Detection of various freshwater cyanobacterial toxins using ultra-performance liquid chromatography-tandem mass spectrometry. *Toxicon* 55:965–972.

Osswald, J., S. Rellan, A. Gogo, and V. Vasconcelos. 2007. Toxicology and detection methods of alkaloid neurotoxin produced by cyanobacteria, anatoxin-a. *Environ. Int.*, 33:1070–1089.

Ouahid, Y., G. Perez-Silva, and F.D. Campo. 2005. Identification of potentially toxic environmental *microcystis* by individual and multiple PCR amplifications of specific microcystin synthetase gene regions. *Environ. Toxicol.*, 20:235–242.

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Pan, H., L. Song, Y. Liu, and T. Borner. 2002. Detection of hepatotoxic *microcystis* strains by PCR with intact cells from both culture and environmental samples. *Arch. Microbiol.*, 178(6):421–427.

Park, H.D., M.F. Watanabe, K. Harada, H. Nagai, M. Suzuki, M. Watanabe, and W.W. Carmichael. 1993. Hepatotoxin (microcystin) and neurotoxin (anatoxin-a) contained in natural blooms and strains of cyanobacteria from Japanese freshwaters. *Nat. Toxins*, 1:353–360.

Poon, G.K., L.J. Griggs, C. Edwards, K.A. Beattie, and G.A. Codd. 1993. Liquid chromatography–electrospray ionization–mass spectrometry of cyanobacterial toxins. *Jour. Chromatogr.*, 628(2):215–233.

Powell, M.W. 1997. Analysis of anatoxin-a in aqueous samples. *Chromatographia*, 45(1):25–28.

Pyo, D., and H. Shin. 1999. Supercritical fluid extraction of microcystins from cyanobacteria. *Anal. Chem.*, 71(20):4772–4775.

Rantala, A., D. Fewer, M. Hisbergues, L. Rouhiainen, J. Vaitomaa, and K. Sivonen. 2004. Phylogenetic evidence for the early evolution of microcystin synthesis. *Proc. Nat. Acad. Sci. USA*, 101(2):568–573.

Rapala, J., K. Sivonen, R. Luukkainen, and S.I. Niemela. 1993. Anatoxin-a concentration in *Anabaena* and *Aphanizomenon* under different environmental conditions and comparison of growth by toxic and non-toxic *Anabaena*-strains: A laboratory study. *Jour. Appl. Phycol.*, 5(6):581–592.

Rapala, J., K. Erkomaa, J. Kukkonen, K. Sivonen, and K. Lahti. 2002a. Detection of microcystins with protein phosphatase inhibition assay, high-performance liquid chromatography–UV detection and enzyme-linked immunosorbent assay: Comparison of methods. *Anal. Chim. Acta*, 466(2):213–231.

Rapala, J., K. Lahti, L.A. Rasanen, A.L. Esala, S.I. Niemela, and K. Sivonen. 2002b. Endotoxins associated with cyanobacteria and their removal during drinking water treatment. *Water Res.*, 36(10):2627–2635.

Rawn, D.F.K., B.P.Y. Lau, B. Niedzwiadek, and J.F. Lawrence. 2005. Food chemical contaminants: Improved method for the determination of anatoxin-a and two of its metabolites in blue-green algae using liquid chromatography with fluorescence detection. *Jour AOAC Int.*, 88(6):1741–1747.

Rawn, D.F.K., B. Niedzwiadek, B.P.Y. Lau, and M. Saker. 2007. Anatoxin-a and its metabolites in blue-green algae food supplements from Canada and Portugal. *Jour. Food Prot.*, 70(3):776–779.

Rinta-Kanto, J.M., and S.W. Wilhelm. 2006. Diversity of microcystin-producing cyanobacteria in spatially isolated regions of Lake Erie. *Appl. Environ. Microbiol.*, 72(7):5083–5085.

Rinta-Kanto, J.M., A.J.A. Ouellette, G.L. Boyer, M.R. Twiss, T.B. Bridgeman, and S.W. Wilhelm. 2005. Quantification of toxic *Microcystis* spp. during the 2003 and 2004 blooms in western Lake Erie using quantitative real-time PCR. *Environ. Sci. Technol.*, 39(11):4198–4205.

Rouhiainen, L., T. Vakkilainen, B.L. Siemer, W. Buikema, R. Haselkorn, and K. Sivonen. 2004. Genes coding for hepatotoxic heptapeptides (microcystins) in the cyanobacterium *Anabaena* strain 90. *Appl. Environ. Microbiol.*, 70(2):686–692.

Sano, T., K. Nohara, F. Shiraishi, and K. Kaya. 1992. A method for the micro-determination of total microcystin content in waterblooms of cyanobacteria (blue-green algae). *Int. Jour. Anal. Chem.*, 49:163–170.

Schèolin, A., A. Siegbahn, L. Lind, C. Berne, G. Sundkvist, E. Bjèork, and F.A. Karlsson. 2004. CRP and IL-6 concentrations are associated with poor glycemic control despite preserved beta-cell function during the first year after diagnosis of Type 1 diabetes. *Diab/Metab. Res. Rev.*, 20(3):205–210.

Schober, E., M. Werndl, K. Laakso, I. Korschineck, K. Sivonen, and R. Kurmayer. 2007. Interlaboratory comparison of Taq nuclease assays for the quantification of the toxic cyanobacteria *Microcystis* sp. *Jour. Microbiol. Methods*, 69(1):122–128.

Siegelman, H.W., N.H. Adams, R.D. Stoner, and D.W. Slatkin. 1984. Toxins of *Microcystis aeruginsoa* and their hematological and histopathological effects. In *Seafood toxins*. E.P. Ragelis, ed. Washington, D.C.: American Chemical Society. pp. 107–113.

Snyder, S., B. Vanderford, P. Roefer, and D. Rexing. 2004. Analysis of algal toxins by LC/MS/MS. In *2004 Water Quality Technology Conference proceedings*. Denver, Colo.: AWWA.

Spoof, L., K. Karlsson, and J. Meriluoto. 2001. High-performance liquid chromatographic separation of microcystins and nodularin, cyanobacterial peptide toxins, on C18 and amide C16 sorbents. *Jour. Chromatogr. A*, 909(2):225–236.

Spoof, L., P. Vesterkvist, T. Lindholm, and J. Meriluoto. 2003. Screening for cyanobacterial hepatotoxins, microcystins and nodularin in environmental water samples by reversed-phase liquid chromatography–electrospray ionisation mass spectrometry. *Jour. Chromatogr.*, 1020(1):105–119.

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Spoof, L., K.A. Berg, J. Rapala, K. Lahti, L. Lepisto, J.S. Metcalf, G.A. Codd, and J. Meriluoto. 2006. First observation of cylindrospermopsin in *Anabaena lapponica* isolated from the boreal environment (Finland). *Environ. Toxicol.*, 21(6):552–560.

Takino, M., S. Daishima, and K. Yamaguchi. 1999. Analysis of anatoxin-a in freshwaters by automated on-line derivatization–liquid chromatography–electrospray mass spectrometry. *Jour. Chromatogr.*, 862(2):191–197.

Tillett, D., D.L. Parker, and B.A. Neilan. 2001. Detection of toxigenicity by a probe for the microcystin synthetase A gene (mcyA) of the cyanobacterial genus *Microcystis*: Comparison of toxicities with 16S rRNA and phycocyanin operon (phycocyanin intergenic spacer) phylogenies. *Appl. Environ. Microbiol.*, 67(6):2810–2818.

Törökné, A., M. Asztalos, M. Bankine, H. Bickel, G. Borbely, S. Carmeli, G.A. Codd, J. Fastner, Q. Huang, A. Humpage, J.S. Metcalf, E. Rabai, A. Sukenik, G. Suranyi, G. Vasas, and V. Weiszfeiler. 2004. Interlaboratory comparison trial on cylindrospermopsin measurement. *Anal. Biochem.*, 332(2):280–284.

Vaitomaa, J., A. Rantala, K. Halinen, L. Rouhiainen, P. Tallberg, L. Mokelke, and K. Sivonen. 2003. Quantitative real-time PCR for determination of microcystin synthetase E copy numbers for *Microcystis* and *Anabaena* in lakes. *Appl. Environ. Microbiol.*, 69:7289–7297.

Via-Ordorika, L., J. Fastner, R. Kurmayer, M. Hisbergues, E. Dittmann, J. Komarek, M. Erhard, and I. Chorus. 2004. Distribution of microcystin-producing and non-microcystin-producing *Microcystis* sp. in European freshwater bodies: Detection of microcystins and microcystin genes in individual colonies. *Syst. Appl. Microbiol.*, 27:592–602.

Westrick, J.A., B. Southwell, and J. Sinclair. 2006. Update on a national preliminary algal toxin occurrence study that monitored source and distribution waters. In *2006 Water Quality Technology Conference proceedings.* Denver, Colo.: AWWA.

Wicks, R.J., and P.G. Thiel. 1990. Environmental factors affecting the production of peptide toxins in floating scums of the cyanobacterium *Microcystis aeruginosa* in a hypertrophic African reservoir. *Environ. Sci. Technol.*, 24:1413–1481.

Yoo, R.S., W.W. Carmichael, R.C. Hoehn, and S.E. Hurdley. 1995. *Cyanobacterial (blue-green algal) toxins: A resource guide.* Denver, Colo.: Awwa Research Foundation and AWWA. p. 229.

Yoshida, M., T. Yoshida, Y. Takashima, N. Hosoda, and S. Hiroishi. 2007. Dynamics of microcystin-producing and non-microcystin-producing *microcystis* populations is correlated with nitrate concentration in a Japanese lake. *FEMS Microbiol. Lett.*, 266(1):49–53.

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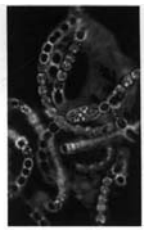

Chapter 4

Algal Chlorophylls: A Synopsis of Analytical Methodologies

David F. Millie, Ryan J. Pigg, Gary L. Fahnenstiel, and Hunter J. Carrick

Assessments of aquatic ecosystem health in response to natural and/or anthropogenic stressors require the quantification and interpretation of relevant, integrative indicators (Millie et al. 2006). Chromophores, including the lipophilic chlorophylls and carotenoids (carotenes and xanthophylls), and the hydrophilic phycobilins (phycocyanin, allophycocyanin, and phycoerythrin), commonly are used as qualitative biomarkers for differentiating algal phylogenetic groups. Chlorophylls and carotenoids are embedded within thylakoid membranes of an algal cell's chloroplasts (or distributed as protein complexes throughout the cytoplasm in photosynthetic cyanobacteria), whereas phycobilin pigments are bound to proteins (biliproteins) adhered to the membranes. Because they capture solar energy and mediate its conversion to chemical energy, chlorophylls, carotenoids, and phycobilins control primary production and ultimately are responsible for regulating carbon and nutrient flux throughout the biosphere (Paerl et al. 2003, 2005).

Light absorbance of all plants is maximal in the blue (400–500 nm) and to a lesser extent, the red (600–700 nm) portions of the visible spectrum, where absorbance by chlorophylls are the greatest (Figure 4-1). Upon excitation by light (photons), electrons of a chlorophyll molecule are raised to a higher energy level, after which the molecule returns to its unexcited "ground" state and releases the potential energy as light (through fluorescence or phosphorescence emission), heat (i.e., nonphotochemical quenching), via transference to a neighboring pigment, or through direct participation in a photochemical reaction (see Figure 4-2; Noggle and Fritz 1976, Whitmarsh and Govindjee 1999). Chlorophyll *a* participates directly in the harvesting and conversion of radiant energy to chemical energy, whereas accessory chlorophylls (e.g., *b*, *c*, *d*),

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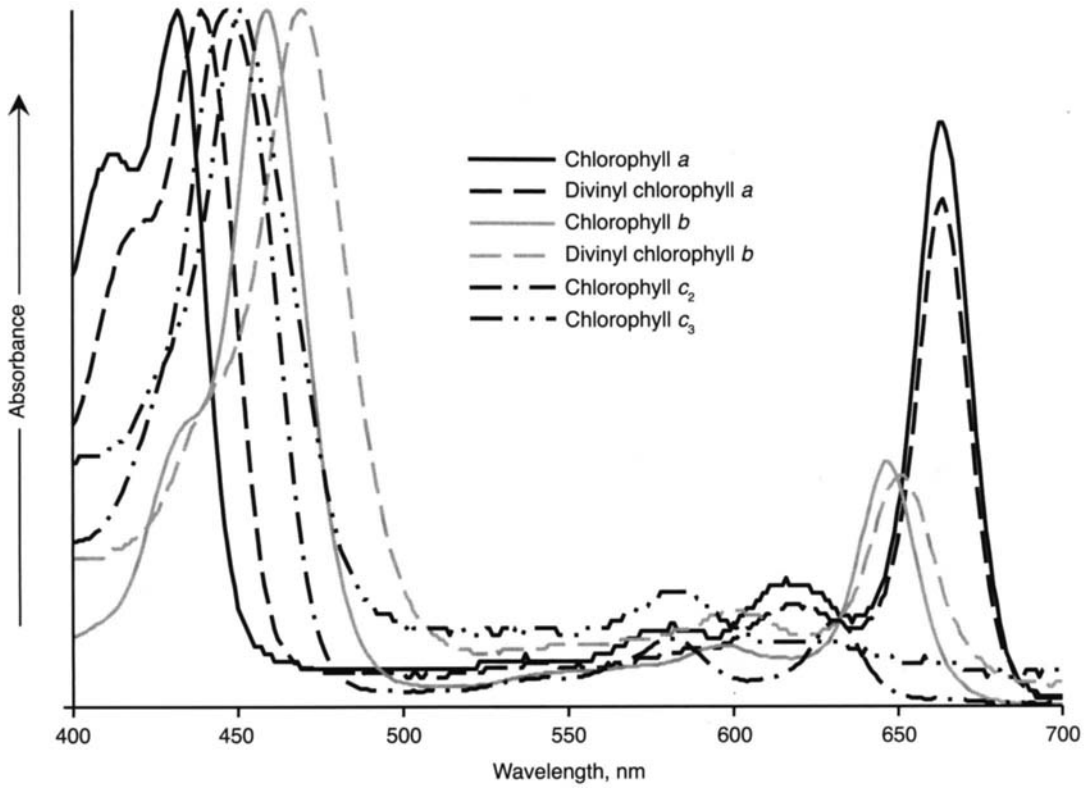

Figure 4-1 *In vitro* absorbance of select chlorophylls distributed among the spectral classes of algae (refer to Table 4-1). Individual spectra, derived from authentic standards, are normalized to their maximum absorbance value. Purified pigments (with the exception of divinyl chlorophyll *b*, provided by Dr. G. Kirkpatrick of Mote Marine Laboratory, Sarasota, Fla., USA) were obtained commercially from the ChromaDex Corporation (Irvine, Calif., USA) and DHI Water & Environment (Hørsholm, Denmark).

select carotenoids, and phycobilins also harvest radiant energy but transfer the excitation energy to chlorophyll *a*. Specifically, light-harvesting pigments within a photosystem's pigment–protein complex transfer energy to specific, reaction-center chlorophyll *a* molecules. These molecules subsequently act as terminal electron acceptors within two distinct photosystems: photosystem I, which is excited by light of 700 nm or less and allows for the reduction of $NADP^+$ to NADPH, and photosystem II, which absorbs light of 680 nm or less and provides for the splitting of the water molecule and subsequent oxygen generation (Figure 4-2). In the cyanobacterium, *Acaryochloris marina* Miyashita et Chihara, chlorophyll *d* comprises greater than 90% of the total chlorophyll (the remainder is chlorophyll *a*) and has replaced chlorophyll *a* in photosystem I (see Miyashita et al. 1997, Larkum and Kühl 2005, Miller et al. 2005).

A survey of the dominant light-harvesting compounds within the spectral classes of eukaryotic algae denotes the diversity of chlorophylls among algal phylogenies: chlorophylls *a*, *c*, carotenoids, and biliproteins—cryptophytes; chlorophylls *a*, *d*, carotenoids, and biliproteins—rhodophytes; chlorophylls *a*, *b*, and carotenoids—chlorophytes, euglenophytes, prasinophytes; chlorophylls *a*, *c*, and carotenoids—diatoms, chrysophytes, raphidophytes, dinoflagellates (see Jeffrey 1980; Jeffrey et al. 1997b, 1999; Wright and Jeffrey 2006). This diversity also extends to the oxygenic photosynthetic prokaryotes, with mono- and divinyl chlorophylls *a*, *b*, and carotenoids present within

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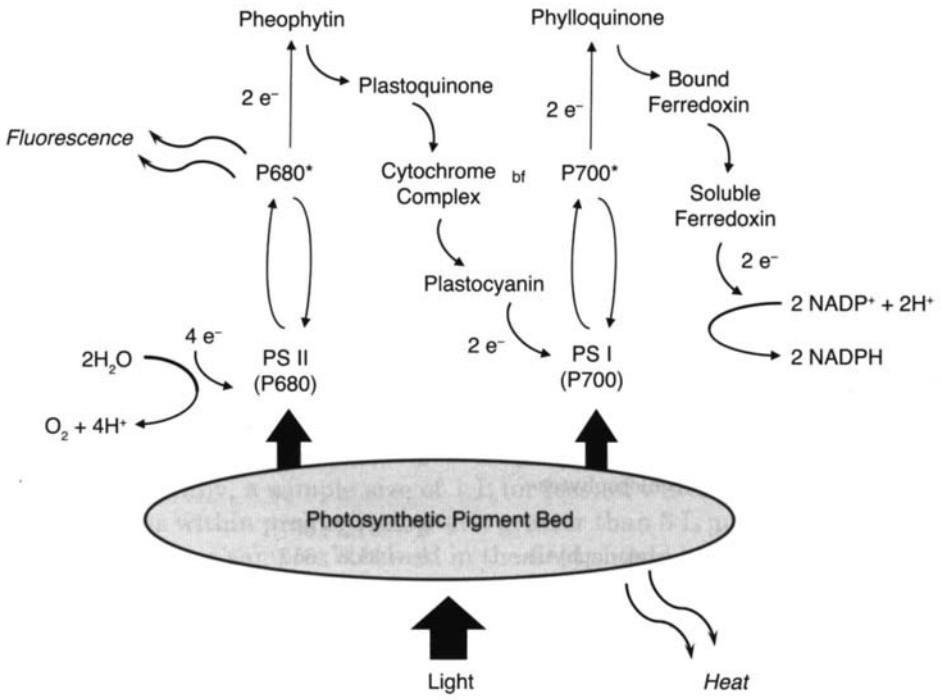

Figure 4-2 Schematic of the light reaction of photosynthesis denoting electron transport within photosystems I and II, following the alteration of P700 and P680 chlorophyll molecules to their excited states (indicated with an asterisk). The site of adenosine triphosphate generation is not diagrammed. (Source: Adapted from Noggle and Fritz 1976, Stryer 1981, and Whitmarsh and Govindjee 1999.)

the prochlorophytes and chlorophyll *a*, carotenoids, and biliproteins in the cyanobacteria (chlorophylls *a* and *d* in *A. marina*). Due to the universal presence of chlorophyll *a* in both eukaryotic and prokaryotic algae, the chlorophyll *a* concentration of a water column (or content per unit substrate) is used as a measurement of total algal biomass. Accordingly, chlorophyll *a*–based assessments are useful in quantifying algal population/assemblage dynamics, physiological state, and growth rate in response to changing environmental variables, comparing assemblage structure among systems, and providing a basis for the understanding of cellular processes and energy cycling (after Harris 1986). Clearly, one's ability to accurately derive and subsequently infer organismal, population, and/or assemblage-level information using such a photopigment biomarker will be dependent upon obtaining significant, detectable, and reliable analytical signals from a particular compound.

Here, we present a synopsis of the sampling and processing procedures, technological approaches, and general applications for the analytical methodologies most commonly utilized for algal chlorophylls. For each of spectrophotometric, fluorometric, and chromatographic methodologies, our evaluation will present the theoretical bases for, the history of methods development in, and the strengths and limitations associated with analytical protocols. Readers are directed to Jeffrey et al. (1997a) and/or appropriate citations listed within each methodological synopsis for detailed procedures and/or comprehensive discussions of the analytical bases that underlie analytical protocols.

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Table 4-1 Occurrence and characteristics of select algal chlorophylls and their derivatives. (Source: Adapted from Jeffrey et al. [1997a], Miyashota et al. [1997a], Aminot and Rey [2000], Ston-Eglert and Kosakowska [2005], and Wright and Jeffrey [2006].)

Chlorophylls & Derivatives	Occurrence	Specific Extinction Coefficient (L g^{-1} cm^{-1}); Maximum Absorbance (nm)	Notes
a	all groups (only select prochlorophytes)	87.67*; 664	monovinyl *a*—a chlorin pigment having a central magnesium ion & a phytol side chain; a proxy for algal abundance
b	chlorophytes, euglenophytes, prasinophytes, prochlorophytes	51.36*; 647	monovinyl *b*
chlorophyllides *a* & *b*	a — all groups; *b* — chlorophytes, euglenophytes, prasinophytes, prochlorophytes	*a* — 127*; 664; *b* — 74.07*; 645	monovinyl *a*/*b* less the phytol chain, senescent processing artifact
pheophytins *a* & *b*	*a* — all groups; *b* — chlorophytes, euglenophytes, prasinophytes, prochlorophytes	*a* — 51.2*; 667; *b* — 31.8*; 657	monovinyl *a*/*b* less the magnesium atom
pheophorbides *a* & *b*	*a* — all groups; *b* — chlorophytes, euglenophytes, prasinophytes, prochlorophytes	*a* — 74.2*; 667; *b* — 46.37*; 657	monovinyl *a*/*b* less the magnesium atom and phytol chain
Divinyl *a* & *b*	prochlorophytes	assume identical to monovinyl chlorophylls	divinyl *a* is a proxy for abundance; determined solely by HPLC
pyropheophytin *a*	all groups	60.29‡; 667	pheophorpide *a* less the methylated carboxyl group from the isocyclic ring
c_1	bacillariophytes, chrysophytes, haptophytes, prymnesiophytes, raphidophytes, phaeophytes, select cryptophytes	44.8†; 631	elutes with chlorophyll c_2 in many HPLC protocols
c_2	bacillariophytes, chrysophytes, cryptophytes, dinophytes, prymnesiophytes, raphidophytes	40.4†; 631	major chlorophyll *c* in chromophytes
c_3	chrysophytes, select bacillariophytes, dinophytes, & prymnesiophytes	335§; 452	
Mg 2,4 divinylpheoporphyrin a_5 monomethyl ester (MGDVP)	prochlorophytes, select prasinophytes and haptophytes, possibly the cyanobacterium, *Acaryochloris marina*	58.9**; 623	
d	trace constituent in rhodophytes; major pigment in the cyanobacterium, *Acaryochloris marina*	N/A; 697**	Differs from chlorophyll *a* by the presence of a formyl group (instead of a vinyl group) at the c-3 position

* 90:10 acetone:water.
† 90:10 acetone:water + 1% pyridine.
‡ diethyl ether
§ acetone + 1% pyridine.
** methanol.

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SAMPLE PROCESSING AND PIGMENT EXTRACTION

Because chlorophyll *a* is used as an estimate of algal biomass, extreme care and adherence to protocols should be undertaken by the scientist to minimize the impact of sample handling and processing upon chlorophyll transformation and/or degradation. Derivatives of chlorophyll (see Table 4-1) can be produced by natural process (such as grazing by heterotrophs, photodegradation, etc., Millie et al. 1993a) as well as artifacts of sample processing. Chlorophyllase is the enzyme responsible for catalyst for the catabolism of chlorophyll *a/b* to chlorophyllide and phytol (Jeffrey and Hallegraeff 1967). Chlorophyllide *a/b* then is transformed (through loss of the magnesium atom) to pheophorbide *a/b*, which later is converted into a primary blue fluorescent catabolite via a red chlorophyll catabolite intermediate (after Hörtensteiner et al. 1998). These degradation products (termed *pheopigments*) are similar structurally to chlorophyll and as such, absorb (and fluoresce) light similarly to that of intact molecules (Wetzel and Likens 2000).

Generally, a sample size of 1 L (or less) of water is sufficient for pigment determinations within productive waters; greater than 3 L may be required for oligotrophic waters. Water samples obtained in the field should be held within acid-rinsed, chemically inert, opaque (or amber) bottles that are properly labeled and kept cool during transport to the laboratory. Processing for chlorophyll within a sample should be completed as soon as possible after sample collection; if samples are to be stored prior to processing, storage should be for no longer than 3 hours (Millie et al. 1993b).

Because photopigments particularly are sensitive to light, heat, oxygen, and acidity (Latasa et al. 2001), laboratory processing of samples should be performed under subdued green light (to minimize damaging absorption by chlorophyll) and in cool conditions (keeping pigment extracts on ice as much as possible), with the scientist using latex/nitrile gloves. Known aliquots of water are drawn under low vacuum (no greater than 5 in. or 12.7 cm Hg) onto precombusted (at 450°C), replicate glass-fiber filters (typically 4.9 $in.^2$ or 17.3 cm^2), after which the filters are folded and blotted to remove excess water. It is generally advised that enough water is filtered to produce a lightly colored filter; a dark color likely will result in a requirement to dilute the pigment extract. Filters (pore size, 0.7 μm) are suitable for capturing microplankton (>20 and <200 μm), most nanoplankton (>2 and <20 μm), and select picoplankton (>0.2 and <2 μm). To improve upon the 0.2-μm cutoff for filtering picoplankton, various filter membranes (see Carrick and Schelske 1997) can be used. However, membrane filters may have substantial shortcomings (including the production of interfering compounds during fluorometric/chromatographic analysis, being extremely brittle, etc.) and should be chosen to best accommodate the chosen analytical technique (from Millie et al. 1993b). If the analysis is not to be completed immediately, filters can placed in appropriate, labeled containers (e.g., cryovials) that are immediately frozen in liquid nitrogen and not allowed to desiccate during storage at –80°C (or less).

Accurate chlorophyll analyses are dependent on optimal extraction of photopigments, and as a consequence, diverse solvents and protocols have been used for extractions. Several criteria for optimal extraction of photopigments are recognized, including complete extraction of all pigments regardless of species composition, stability of pigments within chosen organic solvent, compatibility of the solvent with the desired analytical technique, simplicity for the processing of multiple (to many) samples, and safety in routine laboratory use (after Wright et al. 1997). Aqueous mixtures of acetone and methanol (and combinations thereof) most often have been utilized as extraction solvents for algal pigments. Rowan (1989) noted that an aqueous acetone extract greater than 9:1 acetone:water inhibits the enzyme chlorophyllase, thereby reducing the likelihood of chlorophyll derivatives arising from sample processing. Although

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spectrophotometric and fluorometric analyses generally would not be affected to a great degree by chlorophyllase activity (Jeffrey and Hallegraeff 1967), chlorophyllides will be eluted separately (and quantified distinct) from intact chlorophylls and other chlorophyll derivatives during chromatographic analysis (see Chromatographic Assessment section). Due to improved efficiency of and shorter time requirements for extraction, methanol generally now is recommended as the solvent of choice for extracting most algal pigments (for a detailed assessment, see Wright et al. 1997). However, the choice of extraction solvent should best suit the particular application (i.e., the amount and type of algae in the sample; Speziale et al. 1984). For example, methanol-based mixtures optimize extraction of chlorophyll *b* from chlorophytes and produce sharper chromatographic peaks (more so than acetone-based mixtures; Wright et al. 1997). However, Bowles et al. (1984) reported that chlorophyll was most effectively extracted from diverse taxa by a methanol:acetone (1:1) mixture, yet extraction of chlorophyll *a* from cyanobacteria was optimized with 9:1 acetone:water. Alternative extraction solvents (singularly, in aqueous mixtures, or in mixtures with acetone or methanol) have included ethanol (Jespersen and Christoffersen 1987, Jacobsen and Rai 1990, Aminot and Rey 2000), chloroform (Lloyd and Tucker 1988), glycerol (Millie et al. 1993b), dimethyl sulfoxide (DMSO; Shoaf and Lium 1976, Speziale et al. 1984), and dimethyl formamide (DMF; Speziale et al. 1984, Suzuki and Ishimaru 1990). Speziale et al. (1984) noted that DMF and DMSO–acetone mixtures better extracted chlorophyll pigments from select taxa than did aqueous acetone, whereas Carrick et al. (1993) achieved comparable extraction of chlorophyll *a* with both DMSO and 9:1 acetone:water. Wood (1985) reported a chloroform–methanol mixture (1:1) to be superior to diverse solvents, including DMSO and DMF, for extracting chlorophyll *a* from select laboratory cultures of algae.

If one uses acetone or alcohols, the mechanical grinding of the filters and/or ultrasonic disruption of the cells is an integral part of sample processing. Generally, extraction of 4 to 16 hours at $\leq -20°C$, following the mechanical disruption of the cells, should provide suitable extraction of most lipophilic pigments; one also may soak filters in solvent overnight, prior to sonication or homogenization. The solvent extract then is clarified (easily accomplished using a syringe filter with a pore size of ≤ 0.45 μm or centrifugation at 2,000 to 3,000 rpm for 5 to 10 minutes) prior to spectral measurement (see Spectrophotometric Assessment section). However, the time required for optimal extraction can vary depending upon the species composition and the chosen extraction solvent (Bowles et al. 1984, Buffan-Dubau and Carman 2000). Repetitive freezing and thawing of filters (using liquid nitrogen prior to their immersion in solvent) enhances extraction by disrupting cell walls/membranes, particularly for algae having trichome sheaths and/or rigid cell walls. Buffan-Dubau and Carman (2000) noted that lypholization (freeze-drying) of sample filters significantly improved pigment extraction (when using acetone or buffered methanol as a solvent). An advantage of using DMSO or DMF as an extraction solvent is that grinding or sonication is not required; both solvents are efficient and rapid extractors of photopigments from intact cells at room temperature. Moreover, the absorption coefficients of these solvents are similar to that of acetone; as a consequence, spectrophotometric and fluorometric analyses can be completed even when instrumental calibrations were undertaken using acetone (see next section). Because DMF is a superior extractant and its use allows for the total time required to complete analyses to be less than that when using other solvents (Speziale et al. 1984), the use of DMF for fluorometric and spectrophotometric analyses of chlorophyll is encouraged. However, the toxicity of DMSO (more so than acetone and methanol) and the ease of which both DMSO and DMF are absorbed through the skin, may make these solvents unsuitable for routine use in many laboratories (Wright et al. 1997).

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Independent of the solvent chosen, the water content of the extraction solvent should be kept at a minimum for the extraction protocol. Latasa et al. (2001) noted that loss of pigments (through precipitation) resulted after dilution of extracts to 6:4 acetone:water or less, and 8:2 methanol:water or less, with the greatest losses for chlorophyll at 1:1 acetone:water and 7:3 methanol:water. Initial pigment concentrations remained unchanged after 24 hours only when using 9:1 or 8:2 acetone:water, whereas 60% and 57% of chlorophyll *a* were lost in 1:1 acetone:water and 7:3 methanol:water, respectively. The blotting of filters (immediately following filtration) typically removes a substantial amount of water retained in the filter during sample processing. Any water remaining in the filter will be added to the extraction solvent, thereby altering the chemical makeup of the original solvent composition (particularly when small extraction volumes are used). Pigment extraction using 100% solvent (rather than aqueous mixtures) and/or lypholization of sample filters can help offset problems associated with excess water retention in filters.

SPECTROPHOTOMETRIC ASSESSMENT

Spectrophotometric analysis is the most common (historical and contemporary) protocol used to estimate chlorophyll concentrations from multipigment extracts. The method is based on absorbance measurements of an extracted pigment solution at one or several wavelengths, with the subsequent estimation of chlorophyll concentrations derived through standardized equations incorporating specific extinction coefficients (e.g., Richards and Thompson 1952; Parsons and Strickland 1963; Strickland and Parsons 1972). In addition to quantitative estimates for chlorophylls *a*, *b*, and *c*, (total) plant carotenoids also can be measured from a sample extraction (Strickland and Parsons 1972). In particular, spectrophotometry is the method of preference to quantify purified chlorophyll dissolved within an organic solvent using the Beer–Lambert law (after Mantoura and Repeta 1997):

$$\text{chlorophyll } [\text{g L}^{-1}] = E_{\lambda max}/(\alpha \cdot \text{L})$$

where $E_{\lambda max}$ is the measured absorbance at the wavelength for a pigment's maximum absorbance, α is a chlorophyll's extinction coefficient appropriate to the organic solvent used (L gm^{-1} cm^{-1}, see Table 4-1), and L is the cuvette path length (cm). Purified chlorophylls can be obtained (either in crystalline form, which then must be dissolved within an appropriate solvent, or already dissolved in solvent) from a variety of national and international commercial sources and/or derived from algal cultures with known pigment compositions (see Wright et al. 1991).

Initial steps to develop spectrophotometric-based assessments of algal chlorophylls date to the early 1950s (Richards and Thompson 1952), with a series of later modifications (e.g., Crietz and Richards 1955; Parsons and Strickland 1963; Scientific Committee for Ocean Research, SCOR–United Nations Educational, Scientific and Cultural Organization, UNESCO 1966). Because carotenoids are coextracted with chlorophylls and similarly have absorbance maxima in the blue portion of the spectrum, measurements for distinct chlorophylls are limited to absorbance maxima in the red (Aminot and Rey 2000). The monochromatic method, developed to estimate (solely) chlorophyll *a* in the presence of pheopigment *a*, within aqueous acetone utilizes absorbance at a single wavelength (E_{665}) with an absorbance correction for turbidity (E_{750}), before and after acidification (E_{665a}, E_{750a}), using 1% v/v HCl to produce a pH of 2.6 to 2.8 in the solvent extract (Lorenzen 1967, as modified by Aminot and Rey 2000):

$$\text{chlorophyll } a \ [\mu\text{g L}^{-1}] = 11.4\text{K}\ ((E_{665}-E_{750}) - (E_{665a}-E_{750a}))\ \cdot V_e/(\text{L}\cdot V_f)$$

$$\text{pheopigment } a \ [\mu\text{g L}^{-1}] = 11.4\text{K}\ (\text{R}(E_{665}-E_{750}) - (E_{665a}-E_{750a}))\ \cdot V_e/(\text{L}\cdot V_f)$$

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where L is the cuvette path length (cm), V_e and V_f are extraction and sample volumes (L), respectively, R is the maximum ratio for (E_{665}/E_{665a}, or 1.7), and K is (R/R–1, or 2.43). The trichromatic method, developed to estimate chlorophylls *a*, *b*, and *c* in the absence of degradation products within aqueous acetone, utilizes absorbance at three wavelengths (E_{664}, E_{647}, and E_{630} for chlorophylls *a*, *b*, and *c*, respectively) and an absorbance correction for turbidity (E_{750}; Jeffrey and Humphrey 1975):

$$\text{chlorophyll } a\ [\mu g\ L^{-1}] = 11.85(E_{665}-E_{750}) - 1.54 \cdot (E_{647}-E_{750}) - 0.08 \cdot (E_{630}-E_{750}) \cdot V_e/(L \cdot V_f)$$

$$\text{chlorophyll } b\ [\mu g\ L^{-1}] = -5.43(E_{665}-E_{750}) - 21.03(E_{647}-E_{750}) - 2.66(E_{630}-E_{750}) \cdot V_e/(L \cdot V_f)$$

$$\text{chlorophyll } c\ [\mu g\ L^{-1}] = -1.67(E_{665}-E_{750}) - 7.60(E_{647}-E_{750}) - 24.52(E_{630}-E_{750}) \cdot V_e/(L \cdot V_f)$$

where abbreviations are as above. To avoid underestimations of chlorophyll concentrations, spectrophotometer bandwidth for absorbance measurements should not exceed 2 nm (Aminot and Rey 2000).

Modifications in the mono- and trichromatic protocols generally have focused on improving upon the extinction coefficients used for calculating concentrations and in solvents other than acetone (e.g., methanol; Holm-Hansen and Riemann 1978). Remarkably, a consistency among (estimates of) extinction coefficients for diverse chlorophylls exists, indicating a robust, quantitative quality to the spectrophotometric method. Lorenzen and Jeffrey (1980) evaluated five trichromatic equations using mixtures of purified pigments that mimicked what might commonly occur in oceanic samples. Their recommended (set of) equations accurately predicted the presence and concentrations of chlorophylls *a*, *b*, and *c* among mixtures (<5% error) and produced the lowest error estimates for chlorophyll *c* (variance of 24% to 300%), although accurate estimation of chlorophyll *c* proved problematic for all equations.

The strength of the spectrophotometric method lies in its simplicity, rapid turnover of samples, and flexibility in assaying a variety of pigments. This, coupled with the capital expense required for instrumentation (suitable single-/dual-path spectrophotometers are commonplace in most laboratories or can be purchased for relatively low costs), provides for the routine assessment of chlorophylls by diverse scientists, water quality resource managers, and public-health officials in a variety of environmental applications. However, limitations and/or disadvantages to the quantitative spectrometry of chlorophyll exist. Trichromatic protocols assume the absence of degradation products and as such, calculations can overestimate actual chlorophyll concentrations in the presence of pheopigments. Notably, the presence of chlorophyllide is undetectable in both mono- and trichromatic protocols; it will (erroneously) be reported as an equivalent amount of chlorophyll (Strickland and Parsons 1972). The presence of background color (attributable to dissolved organic compounds) that can interfere with absorbance otherwise attributable to chlorophyll also is ignored.

Moreover, oxygenic, photosynthetic bacteria occur in both marine and freshwater systems. Because bacteriochlorophyll *a* (and its associated phaeophytin product) of the green-sulfur bacteria displays absorbance maxima at or near wavelengths commonly utilized in standardized equations of traditional spectrophotometric protocols (ca. 665 nm), these procedures may misidentify this bacteriochlorophyll as algal-derived chlorophylls and subsequently cause potential misinterpretations of algal distributions and/or rate processes. Curaco and Puccoon (1986) devised spectrophotometric protocols to partition this bacteriochlorophyll from algal chlorophylls; however, Knowlton et al. (1989) provided results proving such an approach problematic and recommended that

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chromatographic protocols (e.g., Hurley and Watras 1991) remain the best methodology for (definitive) assessment of bacteriochlorophylls.

Perhaps the greatest drawback to the spectrophotometric method is its lack of sensitivity; protocols require that relatively large concentrations of pigment be present in samples in order to produce measurable absorbance (i.e., pigment in the extract should be visible; Strickland and Parsons 1972). Low values of chlorophyll absorbance relative to background nontarget compounds are problematic, particularly when using trichromatic approaches where absorbance of chlorophyll *a* at 647 and 630 nm are considerably less than that at 664 nm. To offset this restriction, greater values of absorbance can be achieved using small-volume cuvettes having long path lengths, thereby maximizing instrumental signal to noise (background absorbance) ratio. Greater quantities of cells also can be concentrated onto filters during sample processing, ensuring ample pigment within subsequent extraction solvents. However, the rupturing of cells and/or their passage through the filters can be problematic at the (high) vacuum pressures typically required for filtration of dense accumulations. Extraction artifacts related to this and/or the long time required for optimal extraction of such samples may arise (see Sample Processing and Pigment Extraction section).

FLUOROMETRIC ASSESSMENT

Fluorometry is perhaps the most routine and simplistic method for the analysis of algal chlorophylls. The method initially was developed during the 1960s and 1970s, largely motivated by the interest of the oceanographic community in measuring chlorophyll concentrations using shipboard instruments, and adapted to routine laboratory applications using desktop instrumentation. Later refinements to the general methodology focused on correcting protocols for potential interference of chlorophyll measurements by degradation products and accessory pigments.

In vitro fluorometric assessment is based on the reaction of chlorophyll molecules solubilized in organic solvents and their subsequent excitation by light. Chlorophyll *a* molecules become excited following absorption of high-energy light, after which light of a lesser energy and longer wavelength is emitted (refer to Figure 4-2). Specifically, chlorophyll *a* fluoresces red light when excited by light in the near-ultraviolet, blue, green, or red wavelengths. However, the emitting light may be reabsorbed by chlorophyll *a* and absorption is wavelength dependent (e.g., Figure 4-1). As a consequence, the strength of the fluorescence signal will be dependent on the excitation wavelength. In organic solvents, chlorophyll *a* exhibits maximum absorbance and emission at ca. 440 and 680 nm. In the instrumental detection of chlorophyll, the amount of light emitted is proportional to the chlorophyll concentration within the solvent extract. Fluorometers utilize optical filters to provide distinct excitation and emission wavelengths; an excitation filter allows a select band of light to pass through and excite the chlorophyll molecules (and blocking wavelengths that would allow reflected light to pass), whereas an emission filter allows a select band of the fluoresced light to pass through to the detector (and blocking light at wavelengths that may interfere). Because fluorometers provide a relative measure of fluorescence, instruments must be calibrated (using purified chlorophyll) to provide quantitative estimates of chlorophyll *a* (Wetzel and Likens 2000). The use of a secondary standard (a coproporphyrin or substance that has optical properties and fluoresces at wavelengths similar to that of chlorophyll *a*) is strongly encouraged; with its routine use (i.e., every time fluorometric measurements are completed), instrumental calibration with a purified chlorophyll standard is required only on a semiannual or annual basis.

Compared to spectrophotometric analysis, fluorometry is significantly more sensitive, less time-consuming, and not as dependent on cuvette handling/matching and

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precise wavelength alignment of the instrument (after Holm-Hansen et al. 1965, Smith et al. 1981). As a consequence, fluorometry is preferred for routine chlorophyll assessments in most laboratory and field studies (Wetzel and Likens 2000). However, chlorophyll estimation via fluorometry generally is not as accurate as the spectrophotometric method; fluorometry can over- and underestimate chlorophyll *a* and chlorophyll degradation products, respectively, when large amounts of chlorophyll *c* are present within samples. The reverse occurs when large amounts of chlorophyll *b* are present (Gibbs 1979; Gieskes and Kraay 1982, 1984; Trees et al. 1985). Filtration-derived artifacts can be a serious problem (Carrick and Schelske 1997); however, the sensitivity of the fluorometric technique allows for smaller filtration volumes, thereby eliminating many ancillary problems associated with sample processing. Dissolved organic material that exhibits similar fluorescence properties to chlorophyll *a* also can act as interfering agents within the analysis.

Since Yentsch and Menzel's (1963) application of fluorescence for determining the concentration of chlorophyll in phytoplankton, several *in vitro* protocols for the fluorometric analysis of chlorophyll, both in the absence and presence of degradation pigments, have evolved (see Holm-Hansen et al. 1965, Strickland and Parsons 1972) and generally are similar in their methodologies. In the absence of pheopigments, chlorophyll *a* concentrations can be calculated as (from Arar and Collins 1997):

$$\text{chlorophyll } a\ [\mu g\ L^{-1}] = (F_e \cdot I_r) \cdot V_e \cdot D\ /(10^3 \cdot V_f)$$

where F_e is the fluorescence of the extract, I_r is the instrumental response factor at a distinct sensitivity setting, V_e and V_f are extraction (mL) and filtered (L) volumes, respectively, and D is the extract dilution factor, if any. To allow a quantitative assessment of both chlorophyll *a* and pheopigment *a*, a second fluorometric measurement after sample acidification (using 0.1 N HCl to produce 0.03 N HCl in the extract) is completed as (after Holm-Hansen et al. 1965, from Arar and Collins 1997):

$$\text{chlorophyll } a\ [\mu g\ L^{-1}] = (I_r \cdot (r/(r-1)) \cdot (F_e \cdot F_{e,a}) \cdot V_e \cdot D)\ /(10^3 \cdot V_f)$$

$$\text{phaeophytin } a\ [\mu g\ L^{-1}] = (I_r \cdot (r/(r-1)) \cdot (rF_{e,a} - F_e) \cdot V_e \cdot D)\ /(10^3 \cdot V_f)$$

where r is the ratio of fluorescence for purified chlorophyll *a* before acidification to fluorescence after acidification, $F_{e,a}$ is fluorescence of the extract after acidification, and other abbreviations are as above. Welschmeyer (1994) improved on the accuracy of chlorophyll *a* estimation by using narrow band filters and mercury vapor lamp (to eliminate much of the interferences arising from the presence of accessory chlorophylls *b* and *c*). However, although the elimination of sample acidification (and the subsequent fluorometric measurement) simplified the overall use of this protocol, the ability to measure phaeophytin *a* was eliminated.

For *in vivo* assessments, chlorophyll concentrations are estimated in live cells suspended in water (via excitation/emission characteristics, similar to that of *in vitro* analysis), thereby providing real-time assessment without any time-consuming chemical extraction (Lorenzen 1966, c.f. Strickland 1968). Such an approach allows for the rapid and sensitive measurement of chlorophyll and is useful for large-scale mapping of and/or detecting small-scale temporal/spatial variances in chlorophyll concentrations (e.g., Dandonneau and Neveux 1997, Serodio et al. 2001, Backe-Hansen and Throndsen 2002, Neveux et al. 2003). It must be remembered that *in vivo* fluorescence arises not only from the emission of light from excited chlorophyll *a* molecules, but also that derived (directly or indirectly) from other excited photopigments (dependent on excitation/emission filter set). Because this technique is based on the fluorescence properties of live cells, measured fluorescence not only is dependent on the concentration of chlorophyll, but also the fluorescence yield, which is variable and dependent on species composition, light history and incident irradiation, cell photoadaptative state,

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and temperature (see Falkowski and Raven 1997). As such, *in vivo* measurements likely are more representative of total chlorophyll fluorescence, or even total pigment fluorescence, than simply that of chlorophyll *a* and must be regarded only as rough estimates of chlorophyll *a*. Accordingly, *in vivo* protocols require careful calibration, and a calibration curve generated for any one body of water at a given time may not be applicable to another body of water. The constancy of calibration (or even the use of a secondary standard) cannot always be assumed; it is recommended that whenever *in vivo* fluorescence of chlorophyll is assessed, several samples also be taken for cross-calibration via *in vitro* protocols.

In addition to the (routine) measurement of total chlorophyll, select fluorescence instrumentation provides noninvasive measurements that allow scientists to gauge the efficiency with which absorbed light is utilized photochemically. Because the quantum yield of photosynthesis is determined, in part, by the probability of an absorbed photon being transferred to open functional photosystems, the efficiency of energy transfer from the accessory pigments to the photosystem reaction centers, and the relative balance in the excitation of the two photosystems (Millie et al. 1995b), measurements are highly sensitive to the overall physiological state of the algae and, as a consequence, provide a means to quantify the general health of a population. For example, pulse amplitude modulated (PAM; e.g., Schreiber et al. 1986) fluorometry generates and amplifies a fluorescence signal from algal cells within a whole-water sample via a short pulse of saturating light (reducing only the primary acceptor of photosystem II) whereas fast repetition rate (Kolber et al. 1998, Raateoja 2004) fluorometry generates subsaturating flashlets that cumulatively saturate photosystem II (thereby allowing for repeated charge–separation processes and ultimately, reduction of all electron acceptors; Kromkamp and Forster 2003, Levy et al. 2003). Both fluorometers provide rapid estimates for the maximum quantum yield for stable charge separation at photosystem II (i.e., the ratio of variable fluorescence to maximum fluorescence); however, the two approaches produce distinct maximum fluorescence values and, as result, different values for photochemical efficiency (Kromkamp and Forster 2003). When such measurements are coupled with detailed assessments of the xanthophyll-cycle pigment pool, photophysiological responses to altered light regimes can be characterized (e.g., Schofield et al. 1998, Evens et al. 2001). Through the use of multiple light-emitting diodes in a PAM fluorometer designed specifically for phytoplankton (the Phyto-PAM), high-frequency light pulses can be applied to obtain differential excitation of chlorophyll fluorescence (at 470, 520, 645, and 665 nm). A deconvolution software routine then provides quantum yield estimates for the community and each of the differently pigmented groups (i.e., cyanobacteria, chlorophytes, chromophytes; see Schreiber 1998, Schreiber et al. 2002). When external calibrations for chlorophyll (using taxa representative of the phylogenetic groups of interest) are completed, the relative contribution of individual groups to the total biomass also can be derived.

Flow cytometry is the measurement of individual particles (cells) suspended within a hydrodynamically focused stream of fluid. Applications in phytoplankton ecology initially utilized benchtop or full-sized laboratory hardware adapted from biomedical applications and designed for analyzing small cells. Briefly, algal cells suspended in water pass in single file through an illuminated (by light or lasers) volume where they scatter light and/or fluoresce (via a fluorochrome; e.g., chlorophyll *a*, phycobilins, and/or immunofluorescent probes/stains). The combination of scattered light and emitted fluorescence is collected, filtered, and converted to digital values via detectors, with the specificity of detection controlled by the wavelength selectivity of optical filters and mirrors. This technique allows rapid quantitative measurements of phytoplankton abundance and the capacity to physically sort subpopulations (based on physiological state and cell cycle progression/differentiation) for functional analysis (for a review,

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see Collier 2000). Accordingly, this technology has been instrumental to the detection/enumeration of picoplankton (e.g., Frankel et al. 1990, Marie et al. 1997, Vaulot and Marie 1999) and species of interest (e.g., Vrieling et al. 1997).

Although this particle-derived technology appears to offer several advantages over bulk *in vitro* pigment analysis (including rapid quantification of distinct cell types and *in vivo* characterization of autofluorescence), the equipment costs and expertise required to operate and maintain equipment likely limit its routine application in most monitoring programs (Hofstraat et al. 1994). The recent application of the lab-on-a-chip technology to flow cytometry (Agilent Technologies, Santa Clara, Calif., USA) has led to the development of a small (and relatively inexpensive) microfluidic-based bioanalyzer. The operational utility of chip technology to flow cytometers is apparent; such technology may provide for more routine use of readily adaptable instruments for pigment-based and/or cell-recognition monitoring efforts, and the (already small size of the) bioanalyzer could be reengineered to a sufficient size for inclusion in autonomous underwater vehicles or other *in situ* sampling platforms (see *In Situ* Assessment section).

CHROMATOGRAPHIC ASSESSMENT

Chromatography is the most accurate method for separating and quantifying chlorophylls, chlorophyll derivatives, and carotenoids at extremely low detection levels within monotypic and mixed algal samples. It affords the identification of phylogenetic groups and/or distinct taxa (based on individual or suites of diagnostic pigments) within mixed samples (e.g., Tester et al. 1995; Descy and Métens 1996; Roy et al. 1996; Woitke et al. 1996; Wright et al. 1996; Millie et al. 1995a, 1997, 2002a, 2004); accordingly, it provides an alternative means for enumerating algae, typically completed via tedious microscopic enumeration. Because pigment composition (generally) corresponds with species cell counts or biovolume estimates, natural communities can be characterized with reliability and accuracy. When chemosystematic data is coupled with statistically based modeling approaches (multiple linear regression, e.g., Gieskes and Kraay 1986, Pinckney et al. 1997; simultaneous equations, e.g., Everitt et al. 1990; matrix factorization, e.g., Mackey et al. 1996, 1998; Wright et al. 1996), the absolute/relative contributions of phylogenetic groups (and potentially taxa) to the total chlorophyll concentration/content can be calculated. This (combined) quantitative and qualitative approach facilitates the routine monitoring of community composition within diverse oceanic, lentic, and lotic systems and provides promise that assessments of aquatic biogeochemical flux, primary production, trophic state, water quality conditions, and changes therein can be accomplished across varying spatial and temporal scales (after Millie et al. 1993a).

Pigment chromatography is based on the partitioning of chlorophylls and carotenoids between a polar organic solvent (mobile phase) and a nonpolar substrate (stationary phase). Jeffrey (1961, 1965, 1968, 1974, 1981) pioneered the use of planar chromatography as a means to separate pigments within natural assemblages and laboratory cultures of phytoplankton. In this analysis, a pigment extract is spotted onto paper or an inert substrate (typically glass) coated with a thin layer of adsorbent (e.g., cellulose) and the paper/substrate placed into a container having a small amount of organic solvent, after which chlorophylls and carotenoids separate (according to their differential solubility) within the migrating solvent front. Although one- and two-way paper/thin-layer chromatography is ideal for separating pigments within an extracted pigment suite, quantitative estimations of individual pigments are extremely tedious; following successful separation, individual pigments must be removed from the chromatographic substrate and eluted into an organic solvent, after which concentrations are determined spectrophotometrically using appropriate extinction coefficients (see above; Millie et al. 1993a).

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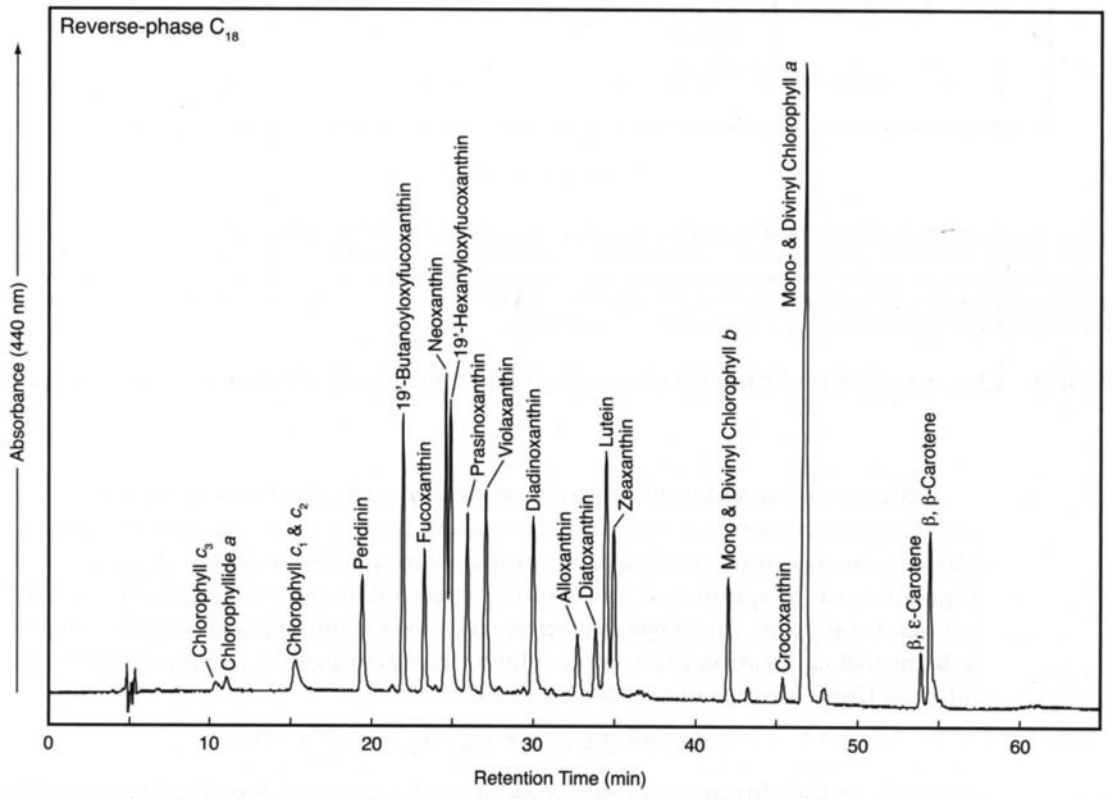
Definitive separation and quantification of chlorophylls and carotenoids within a multipigment mixture is completed via reverse-phase high-performance liquid chromatography (HPLC), incorporating a polar, mobile phase and a nonpolar, stationary phase. In this approach, pigments having the greatest polarity elute first, followed by those of lessening polarities. In a typical analysis, a clarified extract is injected into and passed through a temperature-regulated (typically 40°C) monomeric column packed with irregularly or spherically shaped C_{18}-bonded silica particles via organic (e.g., methanol, acetonitrile) and/or aqueous organic solvents at high pressure. Pigments are attracted to and layer onto the packing particles throughout the column, after which they elute differentially (as polarities of distinct pigments and the mobile phase equalize) and exit the column prior to their detection by in-line spectrophotometers, photodiode array spectrophotometers, and/or fluorometers. To improve chromatographic resolution of early eluting peaks (including chlorophyllides *a* and *b* and chlorophylls $c_{1/2}$ and c_3; see Figures 4-3 and 4-4) within acetone-extracted pigments, Mantoura and Llewellyn (1983) suggested the addition of a buffering agent (tetrabutylammonium acetate and ammonium acetate) to the injected extract. However, Zapata and Garrido (1991) noted such an addition to be unnecessary if a buffered solvent is used in the initial mobile phase.



NOTE: Sample (70:30 standard mix:1.0 M ammonium acetate) was injected into a Model 600E HPLC (Waters Corp., Milford, Mass., USA) equipped with a single monomeric (Microsorb-MV; 100 × 4.6 mm, 3 µm; Varian Inc., Palo Alto, Calif., USA) and a single polymeric (Vydac 201TP, 250 × 4.6, 5 µm; The Separations Group Inc., Hesperia, Calif., USA) reverse-phase C18 columns in series. Chromatogram was generated by an in-line Waters 2996 photodiode array detector. The mobile phase was programmed as (minutes, solvent flow rate—mL min–1, % Solvent A—80:20 methanol:0.5M ammonium acetate/% solvent b—80:20 methanol:acetone): (0, 0.8, 100/0); (12, 1.0, 60/40); (45, 1.25, 0/100); (49, 1.50, 0/100); (52, 1.50 100/0); (58, 0.8, 100/0). The column temperature was held constant at 45°C.

Figure 4-3 Chromatogram of purified chlorophyll and carotenoid standards obtained from DHI Water & Environment, Hørsholm, Denmark

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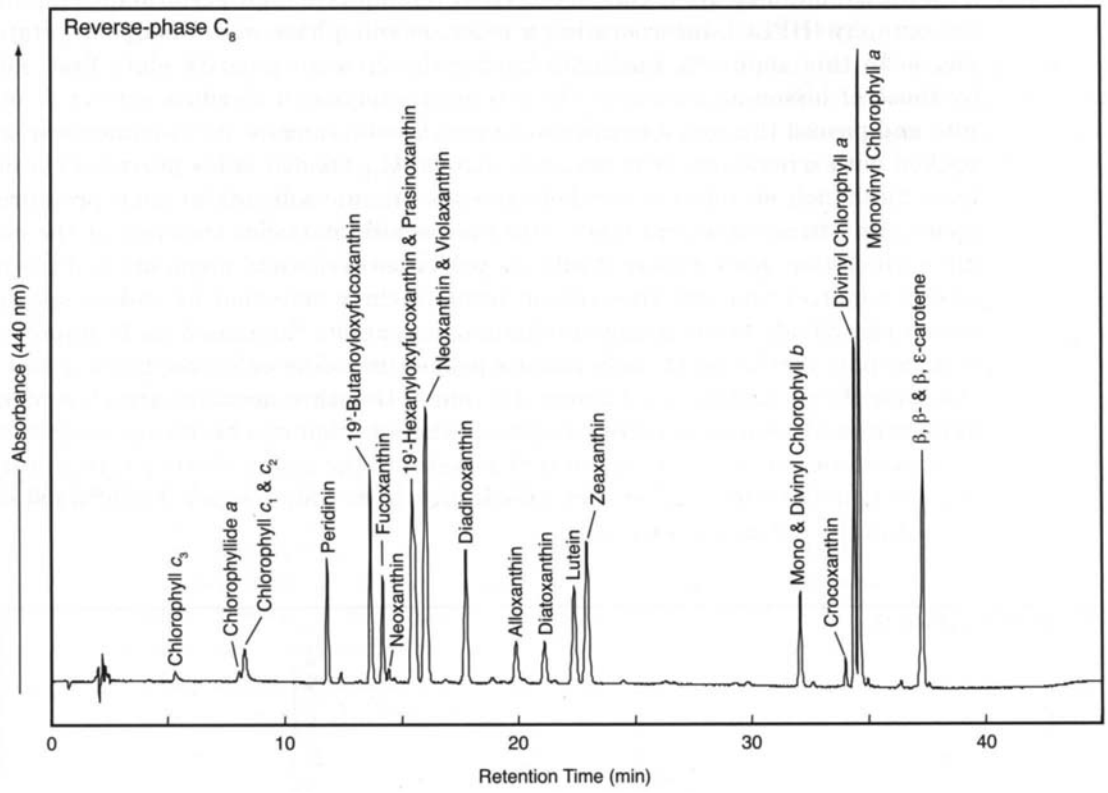

NOTE: Sample (70:30 standard mix:1.0 M ammonium acetate) was injected into a Model 600E HPLC equipped with a MOS-2 Hypersil 150 x 4.6, 3 µm; Thermo Fisher Scientific Inc., Waltham, Mass., USA) C8 column. Chromatogram was generated by an in-line Waters 2996 photodiode array detector. The mobile phase was programmed as (minutes, % Solvent A—70:30 methanol: 1M ammonium acetate/% solvent b—methanol): (0, 75/25); (1, 50/50); (20, 30/70); (25, 0/100); (32, 0/100); (39, 75/25). Solvent flow rate and column temperature were held constant at 1 mL min–1 and 25°C, respectively.

Figure 4-4 Chromatogram of purified chlorophyll and carotenoid standards (refer to Figure 4-3)

After elution, chlorophylls and carotenoids are identified by comparison of chromatographic retention times and absorption spectra (typically determined by in-line photodiode spectrometer and/or scanning fluorometers) to those of known standards. Pigments can be quantified using either external or internal pigment standards. For external standards, response factors are derived for individual pigments via previously determined calibration plots, after which concentrations in sample volumes are calculated as (from Mantoura and Repeta 1997):

$$\text{pigment } [\mu g\ L^{-1}] = (A_p \cdot R_f \cdot (V_e/V_i) \cdot 10^6) / V_f$$

where A_p is the chromatographic peak area at a selected wavelength (typically 436 to 440); R_f is a pigment's response factor (i.e., ng pigment injected per unit peak area); V_e (mL), V_f (L), and V_i (µL) are extraction, sample, and injection volumes, respectively. If a buffer dilution of the sample extract is utilized for injection, the actual volume of pigment extract injected must be corrected for (by the ratio of the extract volume to the total extract and buffer volume).

For internal chemical standards, a known mass of the standard is added to each pigment extract prior to injection, and a relative response factor for each sample pigment to the standard is derived, after which relative sample pigment recoveries are

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calculated (for details and calculations, see Mantoura and Repeta 1997). Synthetic compounds and diverse porphyrins, phorbins, and carotenoids can be used as internal standards; however, care should be taken to select chemicals that are chromatographically distinguishable from naturally occurring pigments and to use compounds with chromatographic signals similar to those of pigments (i.e., strongly absorb and/or fluoresce at ca. 435 and 600 nm, respectively; Mantoura and Repeta 1997). Because internal standards also can be used as a measure of extraction efficiency, sample degradation, and chromatographic reproducibility (Millie et al. 1993b), their use is encouraged.

Technological advances in computer-driven HPLC instrumentation have provided for and improved upon the routine characterization of photopigments. However, the diversity of commercially available HPLC hardware (autosamplers, pumps, columns, detectors, etc.) and supporting computer software, as well as (the range in) costs required to obtain such instrumentation precludes the (practical) standardization of any one instrument. Most importantly, no one chromatographic methodology can resolve all pigments of interest to all scientists (Van Heukelem and Thomas 2001); rather, the needs and/or requirements of individual scientists for analyzing pigments within a distinct set of samples will dictate the appropriate approach. Consequently, a number of chromatographic approaches coupled with judicious use of spectrophotometric and fluorometric detection have been utilized to optimize the separation of chlorophylls and chlorophyll derivatives and carotenoids within algal pigment mixtures (Table 4-2; see Jeffrey et al. 1997a, 1999; Zapata 2005).

Jeffrey (1981) noted that for a chromatographic technique to be useful, it must be rapid, simple to execute, resolve pigments at extremely low levels of detection, be innocuous, and cause no chemical change to the pigments. If one simply is interested in the quantification of chlorophylls *a* and *b* from a pigment extract, pigment separations can be achieved in less than 10 minutes by pumping an isocratic (methanol) mobile phase across a monomeric C_{18} column (Millie et al. 1993b). Incorporating monomeric C_{18} columns into their protocols, Mantoura and Llewellyn (1983) and Wright and Schearer (1984) utilized distinct binary, mobile phases to successfully separate chlorophylls and carotenoids from marine phytoplankton. Wright et al. (1991) proposed a ternary mobile phase to separate 54 chlorophylls, chlorophyll derivatives, and carotenoids in less than 25 minutes from oceanic samples and laboratory cultures of marine phytoplankton. This latter chromatographic protocol is recommended by the SCOR Working Group 78 and UNESCO as the most comprehensive means to separate diverse chlorophylls and carotenoids occurring within marine waters (see Jeffrey et al. 1997a).

Clearly, the important criterion for the successful application of a chromatographic method is its ability to satisfy distinct informational needs. For example, prochlorophytes often dominate the assemblages within the deep chlorophyll-maximum of the open ocean and large lakes, and as such, accurate estimation of their contribution to the assemblage is critical for evaluating production potential and trophic fate within these systems. The monovinyl chlorophylls (3-vinyl-8-ethyl chlorophylls *a* and *b*) are replaced with the divinyl chlorophylls (8-desethyl, 8-vinyl chlorophylls *a* and *b*) in prochlorophytes. Within most chromatographic protocols incorporating a monomeric C_{18} column, mono- and divinyl chlorophylls *a* and *b* co-elute from mixed-algae samples (e.g., Figure 4-3). Chromatographic protocols incorporating monomeric C_8 and C_{30} columns can differentiate the mono- and divinyl chlorophyll *a* (Figure 4-4; see Goericke and Repeta 1992, Barlow et al. 1997, Zapata et al. 2000). In addition, separation of chlorophyll *c* pigments is necessary for accurate, chemosystematic identification and/or quantification of many chromophytic algae (Table 4-1; see Jeffrey et al. 1975, Jeffrey 1989). Chlorophylls c_1 and c_2 co-elute within many chromatographic protocols incorporating a monomeric C_{18} column (Figures 4-3, 4-4; e.g., Mantoura and Llewellyn 1983,

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Table 4-2 Summary and evaluation of analytical methodologies for algal chlorophylls (see text for details)

	Spectrophotometric	Fluorometric	Chromatographic
Principal chlorophylls detected	Monochromatic equations—chlorophyll *a*, pheophytin upon acidification; trichromatic equations—chlorophylls *a*, *b*, *c*	Chlorophyll *a*, pheophytin upon acidification	All chlorophylls (mono- and divinyl, dependent upon methodology); chlorophyll derivatives
Interferences	Dissolved organics (color); bacteriochlorophylls	Fluorescent dissolved organics; accessory chlorophylls (*b*, *c*); chlorophyll degradation products	Chemical artifacts derived from sample processing
Advantages	Simple/rapid measurement of major chlorophylls; inexpensive (requires routine and affordable laboratory instrumentation); simple data reduction	Simple/rapid measurement; high degree of sensitivity (up to 0.01 µg L–1 *in vitro*, 0.02 µg L–1 *in vivo*); simple data reduction	Definitive separation/quantification of chlorophylls; chlorophyll spectra can be generated to confirm identification
Disadvantages	Provides estimate of actual chlorophyll; limited sensitivity due to background absorbance; chlorophyllide is undetectable; can overestimate actual chlorophyll (from trichromatic equation)	Provides relative measure of chlorophyll; Instrumentation is expensive; cannot resolve accessory chlorophylls; routine calibration via purified chlorophyll *a* standard required; *in vivo* measurement sensitive to algal physiology	Instrumentation is very expensive; sample processing is time-consuming—ca. 20 min to 1 hr per analysis; sensitivity dependent upon chromatographic protocol and equipment; routine calibration via purified standards required (limited availability); data reduction is time-consuming and often complex
Recommendations & usage	Recommended for routine analysis of chlorophylls *a*, *b*, *c*; absorption (reflectance) measurement applicable to "passive" remote imagery	Recommended for routine analysis of chl *a* only; *in vivo* protocol provides for repetitive non-invasive measurements, cell counting/sorting (via flow cytometry) and estimates of photochemical efficiency; fluorescence measurement applicable to *in situ* instruments and "active" remote imagery	Recommended for accurate analysis of all photopigments; provides for growth measurement (via ^{14}C-labeling of chlorophyll *a*); applicable to population/community research applications

Wright et al. 1991). Differentiation (of many) of these and other taxonomically distinct chlorophyll *c*–like pigments has been optimized using protocols incorporating monomeric C_8 and high carbon-loaded C_{18} and polymeric C_{18} columns and solvent modifiers, including pyridine (see Kraay et al. 1992, Jeffrey et al. 1999, Zapata et al. 2001).

Using a modified version of Mantoura and Llewellyn's (1983) solvent system and a temperature-dependent, polymeric C_{18} column, Van Heukelem et al. (1992) improved upon pigment separation normally achieved using monomeric columns. In evaluating the temperature dependency of elution profiles of pigments with this column, Van

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Heukelem et al. (1994) noted that intact chlorophylls and chlorophyll derivatives were best separated at 10° to 30°C whereas the optimal separation of carotenoids occurred at 60°C. Notably, monovinyl chlorophyll *a* separated from divinyl *a*, chlorophyll c_1 from c_2, and partial separation of chlorophyll c_2 from Mg 2,4-divinylpheoporphyrin a_5 monomethyl ester (MGDVP), a diagnostic pigment for select prasinophytes. In a combination of the Mantoura and Llewellyn (1983) and Van Heukelem et al. (1992) protocols, Pinckney et al. (1996) used both mono- and polymeric C_{18} columns to enhance separation of closely eluting pigments based on distinct column properties (i.e., the strong retention, high efficiency, and chemical selectivity of monomeric columns and the physical selectivity of polymeric columns; refer to Figure 4-3).

CHLOROPHYLL RADIOLABELING

The labeling of chlorophyll with radioactive carbon (C) can be used to measure rates of chlorophyll synthesis and, given appropriate precautions, estimate phytoplankton growth rate. It is the latter application in which the protocol has been widely used. Given states of balanced growth (i.e., rates of chlorophyll synthesis equal carbon synthesis) and no cell photoadaptation, biosynthesis rates of pigments are equal to rates of C-specific growth. From this, the synthesis of chlorophyll and carotenoids provides for the estimation of *in situ* growth rates of the algal community and/or distinct phylogenetic groups, respectively, in mixed assemblages. Briefly, algae are incubated in the presence of $NaH^{14}CO_3$, and ^{14}C is incorporated into a pool of low-molecular-weight pigment precursors, with the subsequent rate of ^{14}C incorporation into chlorophyll *a* equal that of C incorporation into community biomass. Estimates of community growth rate then are based on quantification of the rate of chlorophyll *a* synthesis during a 24-hour (dawn to dawn) incubation period.

Redalje and Laws (1981) and Redalje (1983) developed techniques for estimating community growth rates via using ^{14}C labeling of chlorophyll *a* and its subsequent separation (from a suite of extracted chlorophylls and carotenoids) via thin-layer chromatography. Gieskes and Kraay (1989) and Gieskes et al. (1993) modified this methodology for HPLC and estimated the contribution of distinct phylogenetic groups to total production through the ^{14}C labeling of chlorophyll *a* and chemosystematic carotenoids. Goericke and Welschmeyer (1992a, 1992b, 1993a, 1993b) utilized pigment radiolabeling to estimate turnover rates for the chlorophyll *a* tetrapyrole ring and its phytol side chain and the biosynthesis and turnover rates for chlorophyll *a* and specific carotenoids, and to assess the assumptions of the ^{14}C-labeling technique. They suggested that strict incubation conditions be used (i.e., 24-hour incubations, light conditions similar to that of *in situ* conditions, etc.) and proposed a new model for determining growth rates from single endpoint experiments.

Typically, ^{14}C labeling of chlorophyll *a* involves the separation and purification of the pigment via HPLC, followed by fraction collection (drop catching) of the pigment eluting from the column and the subsequent quantification of ^{14}C-specific activity via a standalone scintillation counter. However, this approach can be quite complex and tedious; fraction collection of chlorophyll *a* can require the gathering of multiple eluent fractions (when multiple chlorophyll derivatives and/or significant amounts of degradation products are present), and scintillation counting typically requires long times (>1 hour) for counting the low activities associated with individual pigment peaks. Although automated fraction collectors can be incorporated in-line with chromatographic and detection instrumentation, drop catching is impractical as part of a long-term monitoring program (where the analysis of radiolabeled, diagnostic photopigments in numerous samples is completed on a weekly or biweekly basis; e.g., Pinckney et al. 1996, 2001). In devising a practical protocol for the routine assessment of ^{14}C activity

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associated with multiple chemotaxonomic pigments, Pinckney et al. (1996) utilized an in-line radiodetector in which a scintillation cocktail was mixed with the HPLC eluent (downstream from the column) prior to fluorescence-based activity measurements within a small-volume (500-μL) flow cell. In this approach, reliable counts of sufficient sensitivity to measure low activities (<100 d.p.m.) over short (seconds) timescales were accomplished, and the radiograms obtained in real time were used to both identify the source of and quantify the activity of radioisotope peaks.

Chlorophyll *a* radiolabeling provides estimates of growth rate that are insensitive to grazer impacts, respiration rates, and/or variable chlorophyll:C ratios (Redalje 1993). Prior to the implementation of this approach, scientists should be aware that radiolabeling methodology is more difficult than many other procedures and may produce growth rate estimates with significant sources of error, particularly if care is not used to ensure all assumptions of the technique are met (e.g., 24-hour incubation, dawn to dawn). Large incubation volumes (≥1 L) having significant phytoplankton biomass and inoculated with a considerable amount of $NaH^{14}CO_3$ (e.g., ≥100 μ-Ci of $^{14}C\ L^{-1}$) generally are required to ensure pigment quantities with quantifiable amounts of radioactivity. The handling (and subsequent disposal) of such volumes of radioactive liquid waste and contaminated supplies may preclude the use of such methodology in select laboratories. Moreover, contamination of radioisotope peaks by colorless radiolabeled compounds (e.g., lipids, organic solubles) can lead to overestimation of pigment-specific activities, ultimately biasing growth-rate estimates (Goericke 1992; also see Jespersen et al. 1992). Care should be taken that the pigment extract be radiochemically pure, and it is recommended that pigment extracts be purified (by acidification, phase separation, or saponification) prior to measuring radioactivity (Pinckney et al. 1996, 2001). Additionally, phytoplankton growth can be severely unbalanced during a natural photocycle and/or during altered states of photoacclimation (Gieskes 1991; Goericke and Welschmeyer 1993a, 1993b); as a consequence, rates of photopigment biosynthesis and C-specific growth may not be equal, thereby invalidating the approach.

Finally, a numerical solution must be used to estimate growth rates using the kinetics of chlorophyll labeling. The solution proposed by Goericke and Welschmeyer (1993a) is more complex than the equations initially proposed by Redalje and Laws (1981) and Welschmeyer and Lorenzen (1984), in which relatively simple labeling kinetics were assumed. In the authors' experience (from the analysis of several hundred measurements), results derived using Redalje and Laws's equation agree well (± <10%) with results derived using the more complex equation (of Goericke and Welschmeyer).

IN SITU ASSESSMENT

Autonomous collection of invasive samples via a continuous-flow water sampler (or one preprogrammed for timed collection) can be coupled with *in vitro* and *in vivo* chlorophyll analyses to provide for the collection of high-frequency data. Such data, acquired over small timescales (minutes to hours), allow for the detection of environmental conditions potentially not easily resolvable by the invasive sampling protocols of a typical monitoring program (Paerl et al. 2005). However, the recent advances in computer and instrumental technologies have afforded the utilization of *in situ* automated spectrometers/fluorometers (handheld, moored, and designed for profiling and as tow-bodies) that similarly provide high-resolution chlorophyll data (both spatially and temporally). Such instrumentation has become an integral part of ecological analyses and, accordingly, long-term aquatic monitoring programs (e.g., Arsalane et al. 1993; Cunningham 1996; Serodio et al. 1997, 2001; Schofield et al. 1999; Figueroa et al. 2006; Alliance for Coastal Technologies 2007). Many abiotic (e.g., optical properties of the water, false absorbance/fluorescence signals from detrital/dissolved material)

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and biotic (e.g., phytoplankton composition, cell morphology, physiological state) factors compete for the scatter, reabsorption, or alter the path of natural/fluoresced light, thereby impacting the reliability and precision of *in vivo* measurements. Consequently, *in situ* assessments of chlorophyll generally cannot be inferred to be as accurate as in vitro measurements (cf. Gregor and Maršáalek 2004).

Nevertheless, the utility of *in vivo* absorbance and/or fluorescence data for resolving phytoplankton abundance (and potentially, composition) has generated considerable interest, primarily due to the codevelopment of fixed-wavelength instrumentation capable of generating accurate (and reproducible) measurements, along with autonomous, underwater vehicles capable of carrying instrument payloads (see Dybas 2003, Babin et al. 2005). An assortment of instruments that provide complex data streams relating to the apparent (diffuse attenuation, reflectance) and inherent properties (absorption, fluorescence, scatter/backscatter) of the water column are commercially available. However, complex algorithms are required to extract the algal component from the bulk optical signals, and once they are extracted, techniques for estimating the composition and quantity of algae in a heterogeneous assemblage are required (e.g., Schofield et al. 1999, Babin et al. 2005). Problematic to this approach is colored dissolved organic matter that often dominates water's optical properties (particularly in productive and coastal waters), thereby complicating the utility of algorithms to deconvolve optical properties into the constituent end members. Automated, submersible flow cytometers also have been developed for delineating phytoplankton abundance and composition over a range of cell sizes (Sieracki et al. 1998, Sosik et al. 2003).

Moreover, absorbance/fluorescence spectra (e.g., Yentsch and Yentsch 1979, Faust and Norris 1985) generally are similar in shape (due to the overall masking effect of chlorophyll *a*) and their delineation is dependent upon the ability to differentiate portions of the spectra where accessory chlorophylls and carotenoids have the greatest impact. To accomplish this, scientists have utilized diverse statistical techniques, most notably discriminant analysis (Johnsen et al., 1994; Millie et al. 1995a, 1997; Roelke et al. 1999) and derivative transformation (e.g., Bidigare et al. 1989) combined with a similarity algorithm (Millie et al. 1997, 2002b). Appropriate instrumental technologies incorporating such theoretical approaches can provide real-time, *in situ* discrimination of algal assemblages. For example, Kirkpatrick et al. (2000) constructed a flow-through spectrophotometer utilizing derivative transformation of and similarity comparisons among generated spectra, thereby providing for autonomous characterization of spectra. *In situ* deployment of this optical sensor currently is being carried out as a component both of fixed (e.g., tethered buoys, portable vessels) and mobile (e.g., profilers, gliders) platforms (see Dybas 2003).

The measurement of passive spectral reflectance (frequently termed *ocean color*; Figure 4-5) from airborne or satellite platforms provides an alternative means to estimate surface/subsurface algal abundance through the differential absorption and backscatter associated with photosynthetic pigments, particularly chlorophyll *a* (e.g., Yentsch 1983, Sathyendranath 1987, Aiken et al. 1992, Harding et al. 1992, Stumpf 2001). Typically, models for estimating chlorophyll *a* concentrations within clear, oceanic waters utilize reflectance data in the green and blue portions of the spectrum, whereas models for turbid waters (where inorganic/organic particles absorb such light) employ reflectance in the red, with near-red reflectance used for correction of particle scattering (see Millie et al. 1992). Alternatively, active sensors measure the laser-induced *in vivo* fluorescence of chlorophyll *a* and phycobilins (e.g., Schmitz-Peiffer and Graßl 1990, Schmitz-Peiffer et al. 1990). Imagery data can be obtained from (and digital cartographs of algal abundance generated for) large areas in a short time, thereby circumventing many of the problems of large-scale monitoring programs concerning synoptic coverage and data dissemination in near-real time (Sathyendranath and

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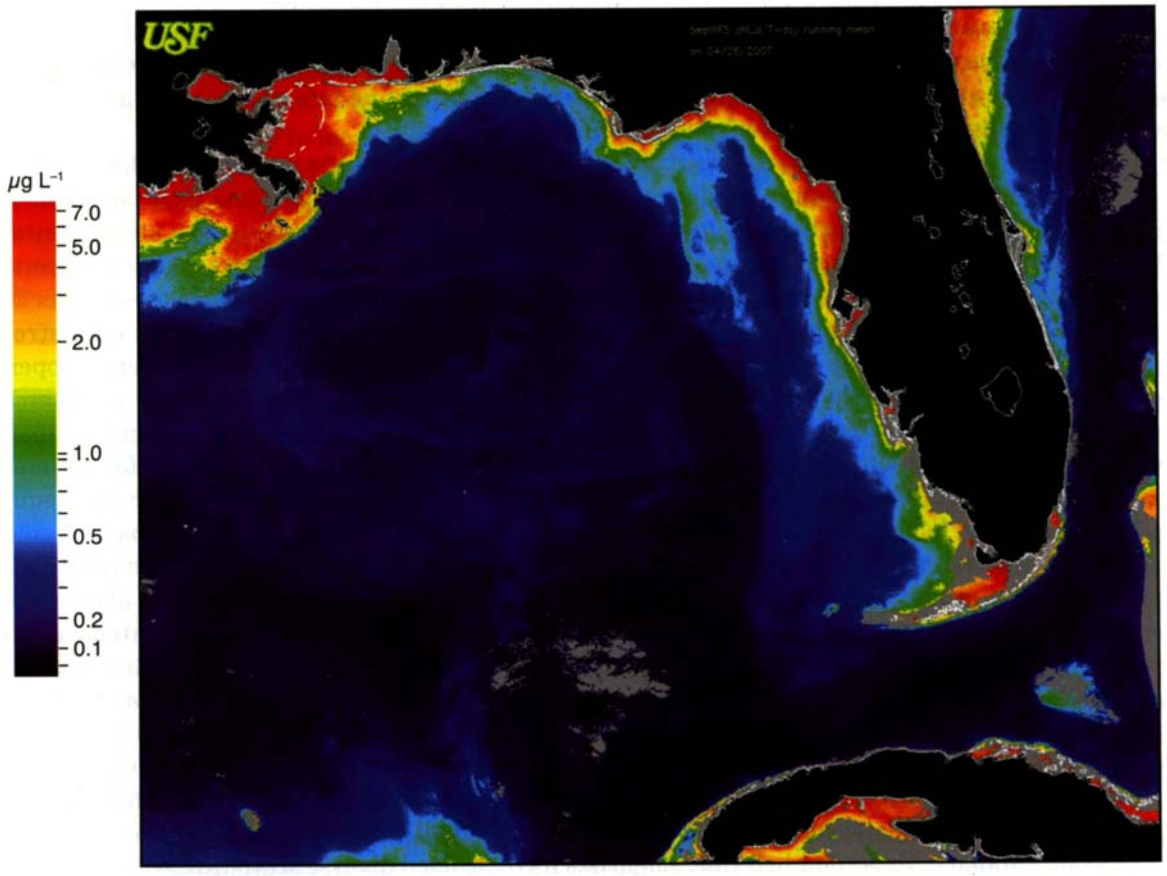

Figure 4-5 Depiction of chlorophyll *a* concentrations throughout the northeastern Gulf of Mexico (seven-day running mean on 26 April 2007). Ocean-color (spatial resolution of 1.1 km) was acquired by the Sea-viewing Wide Field-of-view Sensor (SeaWiFS) orbiting on the SeaStar satellite. Concentrations derived from an algorithm using maximum band ratios incorporating 443, 490, and 510 nm; refer to http://oceancolor.gsfc.nasa.gov/SeaWiFS/BACKGROUND/. (Courtesy of Dr. F. Muller-Karger of the College of Marine Science, University of South Florida, St. Petersburg, Fla., USA.)

Platt 1989). However, chlorophyll estimates derived from remote imagery can be problematic when maximal reflectance/fluorescence signatures attributable to the large amounts of suspended sediments, submerged plants, and/or dense surface scums of bloom-forming phytoplankton arise (e.g., Galat and Verdin 1989; Millie et al. 1992, 1995c). Accordingly, the interpretation of remotely sensed imagery for productive coastal and eutrophic waters is complex (see Sathyendranath et al. 1989), and may be limited in its application. Consequently, airborne/satellite remote sensing may best be suited for the monitoring of chlorophyll *a* in oligotrophic waters, where phytoplankton almost solely determine the optical properties of the water column.

Several factors must be considered when assessing biomass measurements derived from *in situ* estimates of algal pigments. Chlorophyll concentrations derived from ocean color sensors may contain inaccuracies; the large concentrations often apparent in coastal zones and in river plumes likely are overestimates due to colored dissolved organic matter, which absorbs blue light and is confounded with chlorophyll within passive imagery (see Figure 4-5). Moreover, imagery-derived estimates depend on cells being homogeneous with respect to absorptive properties (Morel and Bricaud

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1980). However, absorption/reflectance and fluorescence properties can be variable among algal groups, dependent upon algal pigment content and composition, as well as the cell-packaging effect. Moreover, many algae alter their pigment composition in response to environmental conditions, and/or the assemblage monitored may contain taxa that possess primary light-harvesting pigments other than chlorophyll *a* (e.g., phycobilins). The spatial resolution provided by many *in situ* and airborne sensors affords discrimination of algal biomass as a function of small-scale physical and chemical forcing (e.g., nutrient, salinity, and thermal gradients, wind-streaking of algal surface scums, morphometric effects, tidal fronts), whereas the poorer resolution provided by satellite sensors may not. Nonetheless, the information generated from the aforementioned and newly evolving technologies afford reliable (and desirable) synoptic- to regional-scale mapping of phytoplankton distributions in temporal scales analogous to the abiotic and biotic processes affecting such distributions (see Millie 1995b, 1995c).

SUMMARY AND EVALUATION

Chlorophyll-derived estimates of biomass and growth rates are quantifiers for assessing and comparing the spatial/temporal dynamics and/or the physiological state of algae. As such, they are ideal indicators for coupling population/community structure and function to ecosystem integrity. Fixed-wavelength spectrophotometric absorbance and fluorometric excitation/emission analyses generally are the most popular and simplistic techniques for quantifying chlorophyll *a*. However, these methodologies cannot differentiate among and quantify all individual chlorophylls (and carotenoids) within multipigment mixtures. For that reason, scientists over the last two decades have relied on HPLC for definitive photopigment assessments. Nevertheless, the mainstream application of HPLC for assessing photopigments appears limited, given the sophisticated instrumentation, the required expertise, and the (relatively large) capital investment required for accurate analyses.

Clearly, a singular protocol able to satisfy all researchers' needs (i.e., simplicity, expense, time of analysis, resolution of chlorophyll *a* from accessory chlorophylls, etc.) is neither desirable nor feasible. Protocols for sample collection, sample processing, and subsequent quantification of chlorophylls generally are similar among scientists characterizing algal assemblages within marine, brackish, and freshwater systems. However, different chlorophyll extraction and analysis protocols may result in distinct estimations and/or interpretation of algal abundance under contrasting environmental conditions. Extraction of pigments (including chlorophyll *a*) from cells is variable depending upon the chosen solvents, the composition of the algal assemblage, and the time period allotted for extraction. Cell chlorophyll *a* content differs among phylogentic groups (as well as taxa within a group), and the variability of chlorophyll *a* content by a taxon in response to environmental conditions (particularly light and nutrients) largely is ignored in (total) chlorophyll estimations. Undoubtedly, scientists will utilize a variety of methodologies to satisfy distinct informational needs and equipment and expense limitations/requirements. For example, if rapid chlorophyll analysis is paramount, the use of DMF for pigment extraction, coupled with *in vivo* fluorometric assessment of chlorophyll *a*, is recommended; their use allows for sample filtering, pigment extraction and processing, and analytical protocols to be completed (in their entirety) in less than 40 minutes.

Despite the routine usage and widespread application of any one (or combination of) analytical method(s) for chlorophyll *a*, scientists must be aware of technical and subsequent interpretational problems that may exist. Sample processing, storage, and analytical protocols should be completed in an attempt to minimize the effects of variables known to dramatically influence catabolism of chlorophyll, thereby lessening

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the potential interference of degradation compounds within analytical assessments of the parent compounds. Discrepancies among spectrophotometric estimations may arise due to the variability among published extinction coefficients used within standardized equations and the equations themselves. For fluorometric analyses, potential interferences caused by and/or the inability to resolve accessory chlorophylls (*b* and *c*), chlorophyll derivatives (phaeophytins, pheophorbides, chlorophyllides), and red-shifted chlorophyll *a* are problematic. Variability among fluorescence yields of photosystem II (arising from differences in light fluoresced from distinct taxa and/or a taxon's physiological state and history of light exposure) can impact *in vivo* fluorometric assessments. Spectrophotometric, fluorometric, and select HPLC methodologies also cannot differentiate between monovinyl chlorophyll *a* and divinyl chlorophyll *a;* accordingly, the presence (and importance) of prochlorophytes may be omitted from assessments of select planktonic assemblages). Numerous abiotic/biotic compounds and factors can influence *in situ* absorbance/fluorescence measurements, potentially causing imprecise estimations of actual chlorophyll *a* concentrations. Finally, imagery-derived estimates of algal abundance are based on models whose predictive limits are defined by the accuracy of ground truthing actual chlorophyll concentrations for confirmation and calibration. Therefore, efforts aimed at developing reliable monitoring efforts will require extensive event-response sampling and reliable *in vitro/in vivo* chlorophyll analyses.

It can only be surmised that it is the scientist's obligation to understand thoroughly that a particular methodology may result in distinct quantifications, depending upon the assemblage assessed and/or the nuances of the chosen sample-processing and analytical protocols. Clearly, caution should be exercised when choosing any variable (including photopigments) as a biomarker and the variation of a single (or several) step(s) within chosen sample processing and analytical protocols may be required to optimally characterize ecologically and physiologically diverse algal assemblages. In any event, the routine and reliable quantification of chlorophylls by diverse methodologies provides for the use of these analytes as quantifiers for estimating and characterizing algal abundance and physiological state, respectively.

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REFERENCES

Aiken, J., G.F. Moore, and P.M. Hooligan. 1992. Remote sensing of oceanic biology in relation to global climate change. *Jour. Phycol.*, 28:579–590.

Alliance for Coastal Technologies. 2007. *Enabling sensor interoperability.* ACT 06-07. Cambridge, Md.: University of Maryland Center for Environmental Science, Chesapeake Biological Laboratory.

Aminot, A., and F. Rey. 2000. *Standard procedure for determination of chlorophyll a by spectroscopic methods.* Copenhagen: International Council for the Exploration of the Sea, Techniques in Marine Environmental Sciences.

Arar, E.J., and G.B. Collins. 1997. *US Environmental Protection Agency method 445.0, in vitro determination of chlorophyll a and pheophytin a in marine and freshwater algae by fluorescence, revision 1.2.* Cincinnati, Ohio: US Environmental Protection Agency, National Exposure Research Laboratory, Office of Research and Development.

Arsalane, W., G. Paresys, J.C. Duval, C. Wilhelm, R. Conrad, and C. Buechel. 1993. A new fluorometric device to measure the in vivo chlorophyll a fluorescence yield in microalgae and its use as a herbicide monitor. *Eur. Jour. Phycol.* 28:247–252.

Babin, M., J.J. Cullen, C.S. Roesler, P.L. Donaghay, G.J. Doucette, M. Kahru, M.R. Lewis, C.S. Scholin, M.E. Sieracki, and H.M. Sosik. 2005. New approaches and technologies for observing harmful algal blooms. *Oceanography*, 18:210–227.

Backe-Hansen, P., and T. Throndsen. 2002. Occurrence of pico- and smaller nanoplanktonic flagellates in the inner Oslo fjord, eastern Norway, during the breeding season of the blue mussel (*Mytilus edulis*). *Sarsia*, 87:65–74.

Barlow, R.G., D.G. Cummings, and S.W. Gibb. 1997. Improved resolution of mono- and divinyl chlorophylls *a* and *b* and zeaxanthin and lutein in phytoplankton extracts using reverse phase C-8 HPLC. *Mar. Ecol. Prog. Ser.*, 61:303–307.

Bidigare, R.R., J.H. Morrow, and D.A. Kiefer. 1989. Derivative analysis of spectral absorption by photosynthetic pigments in the western Sargasso Sea. *Jour. Mar. Res.*, 47:323–341.

Bowles, N.D., H.W. Paerl, and J. Tucker. 1984. Effective solvents and extraction periods employed in phytoplankton carotenoid and chlorophyll determinations. *Can. Jour. Fish. Aquat. Sci.*, 42:1127–1131.

Buffan-Dubau, E., and K.R. Carman. 2000. Extraction of benthic microalgal pigments for HPLC analyses. *Mar. Ecol. Prog. Ser.*, 204:293–297.

Carrick, H.J., and C.L. Schelske. 1997. Have we underestimated the importance of small phytoplankton in productive waters? *Limnol. Oceanogr.*, 42:1613–1621.

Carrick, H.J., F.J. Aldridge, and C.L. Schelske. 1993. Wind influences phytoplankton biomass and composition in a shallow, productive lake. *Limnol. Oceanogr.*, 38:1179–1192.

Collier, J.L. 2000. Flow cytometry and the single cell in phycology. *Jour. Phycol.*, 36:628–644.

Crietz, G.I., and F.A. Richards. 1955. The estimation and characterization of plankton populations by pigment analysis. III. A note on the use of millipore membrane filters in the estimation of plankton pigments. *Jour. Mar. Res.*, 14:211–216.

Cunningham, A. 1996. Variability of in vivo chlorophyll fluorescence and its implications for instrument development in bio-optical oceanography. *Sci. Mar.* 60:309–313.

Curaco, N., and A.H. Puccoon. 1986. The measurement of bacterial chlorophyll and algal chlorophyll *a* in natural waters. *Limnol. Oceanogr.*, 31:889–893.

Dandonneau, Y., and J. Neveux. 1997. Diel variations of in vivo fluorescence in the eastern equatorial Pacific: an unvarying pattern. *Deep-Sea Res.*, 44:1869–1880.

Descy, J.-P. and A. Métens. 1996. Biomass-pigment relationships in potamoplankton. *Jour. Plank. Res.*, 18:1557–1566.

Dybas, C.L. 2003. Harmful algal blooms: biosensors provide new ways of detecting and monitoring growing threat in coastal waters. *Bioscience*, 53:918–923.

Evens, T.J., G.J. Kirkpatrick, D.F. Millie, and D. Chapman. 2001. Photophysiological responses of the red-tide dinoflagellate *Gymnodinium breve* (Dinophyceae) under natural irradiance. *Jour. Plank. Res.*, 23:1177–1194.

Copyright (C) 2010 American Water Works Association All Rights Reserved

Everitt, D.A., S.W. Wright, J.K. Volkman, D.P. Thomas, and E.J. Lindstrom. 1990. Phytoplankton community composition in the western equatorial pacific determined from chlorophyll and carotenoid pigment distributions. *Deep-Sea Res.*, 37:975–997.

Falkowski, P.G., and J.A. Raven. 1997. *Aquatic photosynthesis.* Malden, Mass.: Blackwell Sciences.

Faust, M.A., and K.H. Norris. 1985. In vivo spectrophotometric analysis of photosynthetic pigments in matural populations of phytoplankton. *Limnol. Oceanogr.*, 30:1316–1322.

Figueroa, F.L., R. Santos, R. Conde-Alvarez, J.L. Mata, G. Pinchetti, J. Matos, P. Huovinen, A. Schuenhoff, and J. Silva. 2006. The use of chlorophyll fluorescence for monitoring photosynthetic conditions of two tank-cultivated red macroalgae using fish pond effluents. *Bot. Mar.*, 49:275–282.

Frankel, S.L., B.J. Binder, S.W. Chisholm, and H.M. Shapiro. 1990. A high-sensitivity flow cytometer for studying picoplankton. *Limnol. Oceanogr.*, 35:1164–1169.

Galat, D.L. and J.P. Verdin. 1989. Magnitude of blue-green algal blooms in a saline desert lake evaluated by remote sensing: evidence for nitrogen control. *Can. Jour. Fish. Aquat. Sci.*, 45:1959–67.

Gibbs, C.F. 1979. Chlorophyll *b* interference in the fluorometric determination of chlorophyll *a* and "phaeopigments." *Aust. Jour. Mar. Freshwater Res.*, 30:597–606.

Gieskes, W. W. 1991. Algal pigment fingerprints: clue to taxon-specific abundance, productivity, and degradation to phytoplankton in seas and oceans. In *Particle analysis in oceanography. NATO ASI series, Vol. G27.* S. Demers, ed. Berlin: Springer-Verlag.

Gieskes, W.W., and G.W. Kraay. 1982. Comparison of chromatographic (HPLC and TLC) conventional methods for the measurement of chlorophylls in oceanic waters. *Arch. für Hydrobiol. Beiheft. Ergeb. Limnol.*, 16:123.

Gieskes, W.W.C., and G.W. Kraay. 1984. Phytoplankton, its pigments, and primary production at a central North Sea station in May, July and September 1981. *Neth. Jour. Sea Res.*, 18:51–70.

Gieskes, W.W., and G.W. Kraay. 1986. Floristic and physiological differences between the shallow and deep nanophytoplankton community in the eutrophic zone of the open tropical Atlantic revealed by HPLC analysis of pigments. *Mar. Biol.* (Berlin), 91:567–576.

Gieskes, W.W.C., and G.W. Kraay. 1989. Estimating the carbon-specific growth rate of the major algal species groups in eastern Indonesian waters by ^{14}C labeling of taxon-specific carotenoids. *Deep-Sea Res.*, 36:1127–1139

Gieskes, W.W.C, G.W. Kraay, and A.G.J. Buma. 1993. ^{14}C Labeling of algal pigments to estimate the contribution of different taxa to primary production in natural seawater samples. *ICES Mar. Sci. Symp.*, 197:114–120.

Goericke, R. 1992. The chlorophyll-labeling method: the radiochemical purity of chlorophyll *a*—A response to Jespersen et al. 1992. *Jour. Plank. Res.*, 14:1781–1785.

Goericke, R., and D.J. Repeta. 1992. The Pigments of *Prochlorococcus marinus*: The presence of divinyl chlorophyll *a* and *b* in a marine procaryote. *Limnol. Oceanogr.*, 37:425–433.

Goericke, R., and N.A. Welschmeyer. 1992a. Pigment turnover in the marine diatom, *Thalassiosira weissflogii.* I. The $^{14}CO_2$-labeling kinetics of chlorophyll *a*. *Jour. Phycol.*, 28:498–507.

Goericke, R., and N.A. Welschmeyer. 1992b. Pigment turnover in the marine diatom, *Thalassiosira weissflogii.* II. the $^{14}CO_2$-labeling kinetics of carotenoids. *Jour. Phycol.*, 28:507–517.

Goericke, R., and N.A. Welschmeyer. 1993a. The chlorophyll-labeling method: Measuring specific rates of chlorophyll *a* synthesis in cultures and in the open ocean. *Limnol. Oceanogr.*, 38:80–95.

Goericke, R., and N.A. Welschmeyer. 1993b. The carotenoid-labeling method: Measuring specific rates of carotenoid synthesis in natural phytoplankton communities. *Mar. Ecol. Prog. Ser.*, 98:157–171.

Gregor, J., and B. Maršáalek. 2004. Freshwater phytoplankton quantification by chlorophyll *a*: a comparative study of in vitro, in vivo and in situ methods. *Water Res.*, 38:517–522.

Harding, L.W., Jr., E.C. Itsweire, and W.E. Esaias. 1992. Determination of phytoplankton chlorophyll concentrations in the Chesapeake Bay with aircraft remote sensing. *Remote Sens. Environ.*, 40:79–100.

Harris, G.D. 1986. *Phytoplankton ecology: Structure, function and fluctuation.* New York: Chapman & Hall.

Hofstraat, J.W., W.J.M. van Zeijl, M.E.J. de Vreeze, J.C.H. Peeters, L. Peperzak, F. Colijn, and M. Rademaker. 1994. Phytoplankton monitoring by flow cytometry. *Jour. Plank. Res.*, 16:1197–1224.

Copyright (C) 2010 American Water Works Association All Rights Reserved

Holm-Hansen, O., and B. Riemann. 1978. Chlorophyll *a* determination: Improvement in methodology. *Oikos*, 30:438–447.

Holm-Hansen, O., C.J. Lorenzen, R.W. Holmes, and J.D.H. Strickland. 1965. Fluorometric determination of chlorophyll. *Jour. Conseil Perm. Int. Pour L'Explor. Mer*, 30(1):3–15.

Hörtensteiner, S., K.L. Wüthrich, P. Matile, K.-H. Ongania, and B. Kraütler. 1998. The key step in chlorophyll breakdown in higher plants; cleavage of pheophorbide a macrocycle by a monooxygenase. *Jour. Biol. Chem.*, 273:15335–15339.

Hurley, J.P., and C.J. Watras. 1991. Identification of bacteriochlorophylls in lakes via reverse-phase HPLC. *Limnol. Oceanogr.*, 36:307–315.

Jacobsen, T.R., and H. Rai. 1990. Comparison of spectrophotometric, fluorometric and high performance liquid chromatography methods for determination of chlorophyll *a* in aquatic samples: Effects of solvent and extraction procedures. *Int. Rev. Gesamten Hydrobiol.*, 76:207–217.

Jeffrey, S.W. 1961. Paper-chromatographic separation of chlorophylls and carotenoids from marine algae. *Biochem. Jour.*, 80:336–342.

Jeffrey, S.W. 1965. Paper chromatographic separation of pigments in marine phytoplankton. *Aust. Jour. Mar. Freshwater Res.*, 16:307–313.

Jeffrey, S.W. 1968. Quantitative thin-layer chromatography of chlorophylls and carotenoids from marine algae. *Biochim. Biophys. Acta*, 162:271–285.

Jeffrey, S.W. 1974. Profiles of photosynthetic pigments in the ocean using thin-layer chromatography. *Mar. Biol.*, 26:101–110.

Jeffrey, S.W. 1980. Algal pigment systems. In *Primary productivity in the sea.* P.G. Falkowski, ed. New York: Plenum Press.

Jeffrey, S.W. 1981. An improved thin-layer chromatographic technique for marine phytoplankton pigments. *Limnol. Oceanogr.*, 26:191–197.

Jeffrey, S.W. 1989. Chlorophyll *c* pigments and their distribution in the chrompohyte algae. In *The chromophyte algae: Problems and perspectives.* Systematics Association Special Volume 38. J.C. Green, B.C.S. Leadbeater, and W.L. Diver, eds. Oxford: Clarendon Press.

Jeffrey, S.W., and G.M. Hallegraeff. 1967. Chlorophyllase distribution in ten classes of phytoplankton: a problem for chlorophyll analysis. *Mar. Ecol. Prog. Ser.*, 35:293–304.

Jeffrey, S.W., and G.F. Humphrey. 1975. New spectrophotometric equations for determining chlorophylls *a*, *b*, c_1 and c_2 in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pflanzen*, 167:191–194.

Jeffrey, S.W., M. Sielicki, and F.T. Haxo. 1975. Chloroplast pigment patterns in dinoflagellates. *Jour. Phycol.*, 11:374–385.

Jeffrey, S.W., R.F.C. Mantoura, and S.W. Wright (eds.). 1997a. *Phytoplankton pigments in oceanography.* Paris: United Nations Educational, Scientific, and Cultural Organization (UNESCO) Publishing.

Jeffrey, S.W., M. Vesk, and R.F.C. Mantoura. 1997b. Phytoplankton pigments: Windows into the pastures of the sea. *Nat. Res.*, 33:14–29.

Jeffrey, S.W., S.W. Wright, and M. Zapata. 1999. Recent advances in the HPLC pigment analysis of phytoplankton. *Mar. Freshwater Res.*, 50:879–896.

Jespersen, A.-M., and K. Christoffersen. 1987. Measurement of chlorophyll *a* from phytoplankton using ethanol as an extraction solvent. *Arch. für Hydrobiol.*, 109 (3):445–454.

Jespersen, A.-M., J. Nielsen, B. Riemann, and M. Søndergaard. 1992. Carbon-specific phytoplankton growth rates: A comparison of methods. *Jour. Plank. Res.*, 14:637–648.

Johnsen, G., O. Samset, L. Granskog, and E. Sakshaug. 1994. *In vivo* absorption characteristics in 10 classes of bloom-forming phytoplankton: Taxonomic characteristics and responses to photoadaption by means of discriminant and HPLC analysis. *Mar. Ecol. Prog. Ser.*, 105:149–157.

Kirkpatrick, G.J., D.F. Millie, M. Moline, and O. Schofield. 2000. Optical discrimination of a phytoplankton species in natural mixed populations. *Limnol. Oceanogr.*, 45:467–471.

Knowlton, M.F., J.R. Jones, and B.D. Perkins. 1989. A comment on "the measurement of bacterial chlorophyll and algal chlorophyll *a* in natural waters" (Caraco and Puccoon). *Limnol. Oceanogr.*, 34:1381–1382.

Kolber, Z.S., O. Prasil, and P.G. Falkowski. 1998. Measurements of variable chlorophyll fluorescence using fast repetition rate techniques: Defining methodology and experimental protocols. *Biochim. Biophys. Acta*, 1367: 88–106.

Kraay, G.W., M. Zapata, and M.J.W. Veldhuis. 1992. Separation of chlorophylls c_1, c_2, and c_3 of marine phytoplankton by reversed-phase-c18-high-performance liquid chromatography. *Jour. Phycol.*, 28:708–712.

Copyright (C) 2010 American Water Works Association All Rights Reserved

Kromkamp, J., and R. Forster. 2003. The use of variable fluorescence measurements in aquatic ecosystems: Differences between multiple and single turnover measuring protocols and suggested terminology. *Eur. Jour. Phycol.*, 38:103–112.

Larkum, A.W.D., and M. Kühl. 2005. Chlorophyll *d*: the puzzle resolved. *Trends Plant Sci.*, 10:355–357.

Latasa, M., K. van Lenning, J.L. Garrido, R. Scharek, M. Estrada, F. Rodriguez, and M. Zapata. 2001. Losses of chlorophylls and carotenoids in aqueous acetone and methanol extracts prepared for RPHPLC analysis of pigments. *Chromatographia*, 53:385–391.

Levy, O., Z. Dubinsky, and Y. Achituv. 2003. Photobehavior of stony corals: Responses to light spectra and intensity. *Jour. Exper. Biol.*, 206:4041–4049.

Lloyd, S., and C.S. Tucker. 1988. Comparison of three solvent systems for extraction of chlorophyll a from fish pond phytoplankton communities. *Jour. World Aquacult. Soc.*, 19:36–40.

Lorenzen, C.J. 1966. A method for the continuous measurement of *in vivo* chlorophyll concentration. *Deep-Sea Res.*, 13:223–227.

Lorenzen, C.J. 1967. Determination of chlorophyll and pheopigments: Spectrophotometric equations. *Limnol. Oceanogr.*, 12:343–346.

Lorenzen, C.J., and S.W. Jeffrey. 1980. *Determination of chlorophyll in seawater: Report of intercalibration tests.* Technical Papers in Marine Science 35. Paris: United Nations Educational, Scientific, and Cultural Organization (UNESCO) Publishing.

Mackey, M.D., D.J. Mackey, H.W. Higgins, and S. Wright. 1996. CHEMTAX—A program for estimating class abundances from chemical markers: Application to HPLC measurements of phytoplankton. *Mar. Ecol. Prog. Ser.*, 144:265–283.

Mackey, M.D., H.W. Higgins, D.J. Mackey, and D. Holdsworth. 1998. Algal class abundances in the western equatorial Pacific: Estimation from HPLC measurements of chloroplast pigments using CHEMTAX. *Deep-Sea Res.*, 45:1441–1468.

Mantoura, R.F.C., and C.A. Llewellyn. 1983. The rapid determination of algal chlorophyll and carotenoid pigments and their breakdown products in natural waters by reverse-phase high performance liquid chromatography. *Anal. Chem. Acta*, 151:297–314.

Mantoura, R.F.C., and D.J. Repeta. 1997. Calibration methods for HPLC. In *Phytoplankton pigments in oceanography.* S.W. Jeffrey, R.F.C. Mantoura, and S.W. Wright, eds. Paris: United Nations Educational, Scientific, and Cultural Organization (UNESCO) Publishing.

Marie, D., F. Partensky, S. Jacquet, and D. Vaulot. 1997. Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. *Appl. Environ. Microbiol.*, 63:186–193.

Miller, S.R., S. Augustine, T.L. Olson, R.E. Blankenship, J. Selker, and A.M. Wood. 2005. Discovery of a free-living chlorophyll *d*-producing cyanobacterium with a hybrid proteobacterial/cyanobacterial small-subunit rRNA gene. *Proc. Nat. Acad. Sci.*, 102:850–855.

Millie, D.F., M.C. Baker, C.S. Tucker, B.T. Vinyard, and C.P. Dionigi. 1992. High-resolution airborne remote sensing of bloom-forming phytoplankton. *Jour. Phycol.*, 28:281–290.

Millie, D.F, H.W. Paerl, and J.P. Hurley. 1993a. Microalgal pigment assessments using high-performance liquid chromatography: A synopsis of organismal and ecological applications. *Can. Jour. Fish. Aquat. Sci.*, 50:2513–2527.

Millie, D.F., H.W. Paerl, J.P. Hurley, and G.J. Kirkpatrick. 1993b. Algal pigment determinations in aquatic ecosystems: Analytical evaluations, applications, and recommendations. In *Current topics in botanical research.* J. Menon, ed. Trivandrum, India: Council of Scientific Research Integration.

Millie, D.F., G.J. Kirkpatrick, and B.T. Vinyard. 1995a. Relating photosynthetic pigments and *in vivo* optical density spectra to irradiance for the Florida red-tide dinoflagellate, *Gymnodinium breve. Mar. Ecol. Prog. Ser.*, 120:65–75.

Millie, D.F., O.M. Schofield, C.P. Dionigi, and P.B. Johnsen. 1995b. Assessing noxious phytoplankton in aquaculture systems using bio-optical methodologies: A review. *Jour. World Aquacult. Soc.*, 12:329–345.

Millie, D.F., B.T. Vinyard, M.C, Baker, and C.S. Tucker. 1995c. Testing the temporal and spatial validity of site-specific models derived from airborne remote sensing of phytoplankton. *Can. Jour. Fish. Aquat. Sci.*, 52:1094–1107.

Millie, D.F., O. Schofield, G.J. Kirkpatrick, G. Johnsen, P.A. Tester, and B.T. Vinyard. 1997. Detection of harmful algal blooms using photopigment and absorption signatures: A case study of the Florida red-tide dinoflagellate, *Gymnodinium breve. Limnol. Oceanogr.*, 42:1240–1251.

Millie, D.F., G.L. Fahnenstiel, H.J. Carrick, S.E. Lohrenz, and O. Schofield. 2002a.

Copyright (C) 2010 American Water Works Association All Rights Reserved

Phytoplankton pigments in coastal Lake Michigan: Distributions during the spring isothermal period and relation with episodic sediment resuspension. *Jour. Phycol.*, 38:639–648.

Millie, D.F., O. Schofield, G.J. Kirkpatrick, G. Johnsen, and T.J. Evens. 2002b. Using absorbance and fluorescence spectra to discriminate microalgae. *Eur. Jour. Phycol.*, 37:313–322.

Millie, D.F., H.J. Carrick, P.H. Doerong, and K.A. Steidinger. 2004. Intra-annual variability of water quality and phytoplankton within the St. Lucie River Estuary (Florida, USA): A quantitative perspective. *Estuarine Coastal Shelf Sci.*, 61:137–149.

Millie, D.F., G.R. Weckman, H.W. Paerl, J.L. Pinckney, B.J. Bendis, R.J. Pigg, and G.L. Fahnenstiel. 2006. Neural network modeling of estuarine indicators: Hindcasting phytoplankton biomass and net ecosystem production in the Neuse (North Carolina) and Trout (Florida) Rivers. *Ecol. Indic.*, 6:589–608.

Miyashita, H., K. Adachi, N. Kurano, H. Ikemotol, M. Chihara, and S. Miyachi. 1997. Pigment composition of a novel oxygenic photosynthetic prokaryote containing chlorophyll *d* as the major chlorophyll. *Plant Cell Physiol.*, 38:274–281.

Morel, A., and A. Bricaud. 1980. Theoretical results concerning the optics of phytoplankton, with special reference to remote sensing applications. In *Oceanography from space.* J.F.R. Gower, ed. New York: Plenum Press.

Neveux, J., C. Dupouy, J. Blanchot, A. LeBouteiller, M.R. Landry, and S.L. Brown. 2003. Diel dynamics of chlorophylls in high-nutrient, low chlorophyll waters of the equatorial Pacific (180°): Interactions of growth, grazing, physiological responses, and mixing. *Jour. Geophys. Res.*, 108:C12.

Noggle, G.R., and G.J. Fritz. 1976. *Introductory plant physiology.* Englewood Cliffs: Prentice-Hall.

Paerl, H.W., L.M. Valdes, J.L. Pinckney, M.F. Piehler, J. Dyble, and P.H. Moisander. 2003. Phytoplankton photopigments as indicators of estuarine and coastal eutrophication. *Bioscience*, 53:953–964.

Paerl, H.W., J. Dyble, J.L. Pinckney, L.M. Valdes, D.F. Millie, P.H. Moisander, J.T. Morris, B. Bendis, and M.F. Piehler. 2005. Using microalgal indicators to assess human and climactically-induced ecological change in estuaries. In *Estuarine Indicators.* S.A. Bortone, ed. Boca Raton, Fla.: CRC Press.

Parsons, T.R., and J.D. Strickland. 1963. Discussion of spectrophotometric determination of marine-plant pigments, with revised equations of ascertaining chlorophylls and carotenoids. *Jour. Mar. Res.*, 21:155–163.

Pinckney, J.L., D.F. Millie, K.E. Howe, H.P. Paerl, and J.P. Hurley. 1996. Flow scintillation counting of ^{14}C-labeled microalgal photopigments. *Jour. Plank. Res.*, 18:1867–1880.

Pinckney, J.L., T.L. Richardson, D.F. Millie, and H.P. Paerl. 2001. Application of photopigment biomarkers for quantifying microalgal community composition and *in situ* growth rates. *Biogeochem. Estuaries*, 32:585–595.

Pinckney, J.P., D.F. Millie, B.T. Vinyard, and H.P. Paerl. 1997. Environmental controls of phytoplankton bloom dynamics in the Neuse River Estuary (North Carolina, USA). *Can. Jour. Fish. Aquat. Sci.*, 54:2491–2501.

Raateoja, M.P. 2004. Fast repetition rate fluorometry (FRRF) measuring phytoplankton productivity: A case study at the entrance to the Gulf of Finland, Baltic Sea. *Boreal Environ. Res.*, 9:263–276.

Redalje, D.G. 1983. Phytoplankton carbon biomass and specific growth rates determined with the labeled chlorophyll technique. *Mar. Ecol. Prog. Ser.*, 11:217–225.

Redalje, D.G. 1993. The labeled chlorophyll *a* technique for determining photoautotrophic carbon specific growth rates and carbon biomass. In *Current methods in aquatic microbial ecology.* P.F. Kemp, E.F. Sherr, and J.J. Cole, eds. Boca Raton, Fla.: Lewis Publishers. pp. 563–572.

Redalje, D.G., and E.A. Laws. 1981. A new method for estimating phytoplankton growth rates and carbon biomass. *Mar. Biol.*, 62:73–79.

Richards, F.A., and T.G. Thompson. 1952. The estimation and characterization of plankton populations by pigment analyses. II. A spectrophotometric method for estimation of plankton pigments. *Jour. Mar. Res.*, 11:156–171.

Roelke, D.L., C.D. Kennedy, and A.D. Weidemann. 1999. Use of discriminant and fourth-derivative analyses with high resolution absorption spectra for phytoplankton research: limitations at varied signal to noise ratio and spectral resolution. *Gulf Mex. Sci.*, 17:75–86.

Rowan, K.S. 1989. *Photosynthetic pigments of algae.* Cambridge, UK: Cambridge University Press.

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Roy, S., J.-P. Chanut, M. Gosselin, and T. Sime-Ngando. 1996. Characterization of phytoplankton communities in the lower St. Lawrence Estuary using HPLC-detected pigments and cell microscopy. *Mar. Ecol. Prog. Ser.*, 142:55–73.

Sathyendranath, S. 1987. Remote sensing of phytoplankton: A review, with special reference to picoplankton. *Can. Bull. Fish. Aquat. Sci.*, 214:561–587.

Sathyendranath, S., and T. Platt. 1989. Remote sensing of ocean chlorophyll: Consequence of non-uniform pigment profile. *Appl. Optics*, 28:490–5.

Sathyendranath, S., L. Prieur, and A. Morel. 1989. A three-component model of ocean colour and its application to remote sensing of phytoplankton pigments in coastal waters. *Int Jour. Remote Sens.*, 10:1373–1394.

Schmitz-Peiffer, A., and H. Graßl. 1990. Remote sensing of coastal waters by airborne lidar and satellite radiometer. Part 1: A model study. *Int. Jour. Remote Sens.*, 11:2163–2184.

Schmitz-Peiffer, A., T. Viehoff, and H. Graßl. 1990. Remote sensing of coastal waters by airborne lidar and satellite radiometer. Part 2: Measurements. *Int. Jour. Remote Sens.*, 11:2185–2204.

Schofield, O.M., T.J. Evens, and D.F. Millie. 1998. Photosystem II quantum yields and xanthophyll-cycle pigments of *Sargassum natans* (Phaeophyta): dynamic responses under natural sunlight. *Jour. Phycol.*, 34:104–112.

Schofield, O., J. Grzymski, P.W. Bisset, G. Kirkpatrick, D.F. Millie, M.A. Moline, and C.S. Roesler. 1999. Optical monitoring and forecasting systems for harmful algal blooms: Possibility or pipe dream? *Jour. Phycol.*, 35:1476–1496

Schreiber, U. 1998. Chlorophyll fluorescence: new instruments for spectral applications. In *Photosynthesis: Mechanisms and effects*, Vol. V. G. Garab, ed. Dordrecht: Kluwer Publishers.

Schreiber, U., U. Schliwa, and B. Bilger. 1986. Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynth. Res.*, 10:51–62.

Schreiber, U., P. Bird, P.J. Ralph, A.W.D. Larkum, and M. Kühl. 2002. Apparent light requirement for activation of photosynthesis upon rehydration of desiccated beachrock microbial mats. *Jour. Phycology*, 38:125–134.

SCOR-UNESCO. 1966. *Determination of photosynthetic pigments in sea water*. UNESCO Monographs on Oceanographic Methodology, 1. Paris: United Nations Educational, Scientific, and Cultural Organization (UNESCO) Publishing.

Serodio, J., J.M. Silva, and F. Catarino. 1997. Nondestructive tracing of migratory rhythms of intertidal benthic microalgae using *in vivo* chlorophyll *a* fluorescence. *Jour. Phycol.*, 33:542–553.

Serodio, J., J.M. Da Silva, and F. Catarino. 2001. Use of *in vivo* chlorophyll a fluorescence to quantify short-term variations in the productive biomass of intertidal microphytobenthos. *Mar. Ecol. Prog. Ser.*, 218:45–61.

Sieracki, C.K., M.E. Sieracki, and C.S. Yentsch. 1998. An imaging in-flow system for automated analysis of marine microplankton. *Mar. Ecol. Prog. Ser.*, 168:285–296.

Shoaf, W.T., and B.W. Lium. 1976. Improved extraction of chlorophyll *a* and *b* from algae using dimethyl sulfoxide. *Limnol. Oceanogr.*, 21:926–928.

Smith, R.C., K.S. Baker, and P. Dustan. 1981. *Fluorometric technique for the measurement of oceanic chlorophyll in the support of remote sensing*. Report 81-17. La Jolla, Calif.: Scripps Institute of Oceanography, University of California–San Diego.

Sosik, H.M., R.J. Olsen, M.G. Neubert, A.A. Shalapyonok, and A.R. Solow. 2003. Growth rates of coastal phytoplankton from time-series measurements with a submersible flow cytometer. *Limnol. Oceanogr.*, 48:1756–1765.

Speziale, B.J., S.P. Schreiner, P.A. Giammatteo, and J.E. Schindler. 1984. Comparison of n,n-dimethylformamide, dimethylsulfoxide, and acetone for extraction of phytoplankton chlorophyll. *Can. Jour. Fish. Aquat Sci.*, 41:1519–1522.

Stoń-Egiert, J., and A. Kosakowska. 2005. RP-HPLC determination of phytoplankton pigments-comparison of calibration results for two columns. *Mar. Biol.*, 147:251–260.

Strickland, J.D.H. 1968. Continuous measurement of *in vivo* chlorophyll: A precaution. *Deep-Sea Res.*, 15:225–227.

Strickland, J.D.H., and T.R. Parsons. 1972. *A practical handbook of seawater analysis*. 2nd ed. Ottawa: Fisheries Research Board of Canada. p. 1–310.

Stryer, L. 1981. *Biochemistry*. 2nd ed. San Francisco: W.H. Freeman.

Stumpf, R.P. 2001. Applications of satellite ocean color sensors for monitoring and predicting harmful algal blooms. *Hum. Ecol. Risk Assess.*, 7:1363–1368.

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Suzuki, R., and T. Ishimaru. 1990. An improved method for the determination of phytoplankton chlorophyll using N,N-dimethylformamide. *Jour. Oceanogr. Soc. Jpn.*, 46:190–194.

Tester, P.A., M.R. Geesey, C. Guo, H.W. Paerl, and D.F. Millie. 1995. Evaluating phytoplankton dynamics in the Newport River Estuary (North Carolina, USA.) by HPLC-derived pigment profiles. *Mar. Ecol. Prog. Ser.*, 124:237–245.

Trees, C.C., M.C. Kennicutt II, and J.M. Brooks. 1985. Errors associated with the standard fluorometric determination of chlorophylls and pheopigments. *Mar. Chem.*, 17:1–12.

Van Heukelem, L., and C.S. Thomas. 2001. Chromatography modeling software in pigment method development: An evaluation of eleven HPLC columns. *Jour. Chromatogr.*, 910:31–49.

Van Heukelem, L., A.J. Lewitus, T.M. Kana, and N.E. Craft. 1992. High-performance liquid chromatography of phytoplankton pigments using a polymeric reversed-phase C_{18} column. *Jour. Phycol.*, 28:867–872.

Van Heukelem, L., A.J. Lewitus, T.M. Kana, and N.E. Craft. 1994. Improved separations of phytoplankton pigments using temperature-controlled high-performance liquid chromatography. *Mar. Ecol. Prog. Ser.*, 114:303–313.

Vaulot, D., and D. Marie. 1999. Diel variability of photosynthetic picoplankton in the equatorial Pacific. *Jour. Geophys. Res.*, 104:3297–3310.

Vrieling, E.G., W.H. van de Poll, G. Vriezekolk, and W.W.C. Gueskes. 1997. Immuno-flow cytometric detection of the ichthyotoxic dinoflagellates *Gyrodinium aureolum* and *Gymnodinium nagasakiense*. Independence of physiological state. *Neth. Jour. Sea Res.*, 37:91–100.

Welschmeyer, N.A. 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. *Limnol. Oceanogr.*, 39:1985–1992.

Welschmeyer, N.A., and C.J. Lorenzen. 1984. Carbon-14 labeling of phytoplankton carbon and chlorophyll *a* carbon: Determination of specific growth rates. *Limnol. Oceanogr.*, 29:135–145.

Wetzel, R.G., and G.E. Likens. 2000. *Limnological analyses*. 3rd ed. New York: Springer-Verlag.

Whitmarsh, J., and Govindjee. 1999. The photosynthetic process. In *Concepts in photobiology: Photosynthesis and photomorphogenesis*. G.S. Singhal, G. Renger, K-D. Irrgang, S. Sopory and Govindjee, eds. Dordrecht: Kluwer Academic Publishers and Narosa Publishers.

Woitke P., T. Schiwietz, K. Teubner, and J.G. Kohl. 1996. Annual profiles of photosynthetic pigments in four freshwater lakes in relation to phytoplankton counts as well as to nutrient data. *Arch. für Hydrobiol.*, 137:363–384.

Wood, L.W. 1985. Chloroform-methanol extraction of chlorophyll *a*. *Can. Jour. Fish. Aquat. Sci.*, 42:38–43.

Wright, S.W., and S.W. Jeffrey. 2006. Pigment markers for phytoplankton production. In *Marine organic matter: Biomarkers, isotopes and DNA, handbook of environmental chemistry*, Volume 2, Part N. J. Volkman, ed. Berlin: Springer-Verlag.

Wright, S.A., and J.D. Shearer. 1984. Rapid extraction and high-performance liquid chromatography of chlorophylls and carotenoids from marine phytoplankton. *Jour. Chromatogr.*, 294:281–295.

Wright, S.W., S.W. Jeffrey, R.F.C. Mantoura, C.A. Llewellyn, T. Bjørnland, D. Repeta, and N. Welschmeyer. 1991. Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. *Mar. Ecol. Prog. Ser.*, 77:183–196.

Wright, S.W., D.P. Thomas, H.J. Marchant, H.W. Higgins, M.D. Mackey, and D.J. Mackey. 1996. Analysis of phytoplankton of the Australian sector of the Southern Ocean: Comparisons of microscopy and size frequency data with interpretations of pigment HPLC data using 'CHEMTAX' matrix factorization program. *Mar. Ecol. Prog. Ser.*, 144:285–98.

Wright, S.W., S.W. Jeffrey, and R.F.C. Mantoura. 1997. Evaluation of methods and solvents for pigment extraction. In *Phytoplankton pigments in oceanography*. S.W. Jeffrey, R.F.C. Mantoura, and S.W. Wright, eds. Paris: United Nations Educational, Scientific, and Cultural Organization (UNESCO) Publishing.

Yentsch, C.S. 1983. Remote sensing of biological substances. In *Remote sensing applications in marine science and technology*. A.P. Cracknell, ed. Dordrecht: Reidel Publushing Company.

Yentsch, C.S., and D.M. Menzel. 1963. A method for the determination of phytoplankton chlorophyll and pheophytin by fluorescence. *Deep-Sea Res.*, 10:221–231.

Yentsch, C.S., and C.M. Yentsch. 1979. Fluorescence spectral signatures: The characterization of phytoplankton populations by the use of excitation and emission spectra. *Jour. Mar. Res.*, 37:471–483.

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Zapata, M. 2005. Recent advances in pigment analysis as applied to picophytoplankton. *Vie Milieu*, 55:233–248.

Zapata, M., and J.L. Garrido. 1991. Influence of injection conditions in reversed-phase high-performance liquid chromatography of chlorophylls and carotenoids. *Chromatographia*, 31:589–594.

Zapata, M., F. Rodriguez, and J.L. Garrido. 2000. Separation of chlorophylls and carotenoids from marine phytoplankton: A new HPLC method using a reversed phase C-8 column and pyridine-containing mobile phases. *Mar. Ecol. Prog. Ser.*, 195:29–45.

Zapata, M., B. Edvardsen, F. Rodriguez, M.A. Maestro, and J.L. Garrido. 2001. Chlorophyll c2 monogalactosyldiacyglycerdie ester (chl c2-MGDG). A novel pigment for *Chrysochromulina* species (Haptophyta). *Mar. Ecol. Prog. Ser.*, 219:85–98.

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Section II

The Organisms

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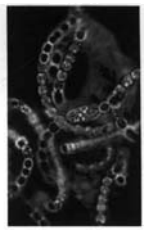

Chapter 5

Cyanobacteria

Andrew D. Chapman

BIOLOGY

Cyanobacteria (blue-green algae, cyanophytes, cyanoprokaryotes) are photosynthetic prokaryotes found in aquatic and terrestrial habitats throughout the world (Komárek 2003a). Cyanobacteria are found in an astonishing array of habitats ranging from Antarctic lakes to hot desert soils (Fogg et al. 1973). They can be found in fresh, brackish, and marine waters; in hot springs (Castenholz 1969); and as lichen photobionts (Brodo et al. 2001). Some cyanobacteria species commonly form surface blooms (Figure 5-1) that can adversely impact water treatment and supply due to filter clogging, increased operational costs, taste and odor problems, and toxin production (Bartram et al. 1999, Oliver and Ganf 2000).

Like other prokaryotes, cyanobacteria lack membrane-bound organelles including nuclei, chloroplasts, and mitochondria and have cell walls composed of peptidoglycan covered by a layer of lipopolysaccharide (Graham and Wilcox 2000). Unlike other photosynthetic bacteria, cyanobacteria possess chlorophyll-*a* (chl-*a*) as their main photopigment. Other forms of chlorophyll (chl-*b*, chl-*c*, and chl-*d*) have been found in a small number of cyanobacteria (Graham and Wilcox 2000). Cyanobacteria may also have a number of accessory pigments including phycobilins (phycocyanin, allophycocyanin, and phycoerythrin) and carotenoids (xanthophylls and β-carotene). Accessory pigments broaden the range of wavelengths that can be used in photosynthesis (Graham and Wilcox 2000) and are responsible for the array of observed colors in cyanobacteria (i.e., blue-green, olive, reddish, black).

Cyanobacteria are morphologically diverse, ranging from unicellular to colonial to filamentous (Whitton and Potts 2000) (Figure 5-2). Colonies can consist of a few cells to many thousands of cells. Cells in colonies can be arranged in regular (i.e., cubic, radial) or irregular patterns (Komárek 2003a). Filaments can be straight, wavy, or coiled; branched or unbranched; and may be solitary or grouped in colonies. Many cyanobacteria have mucilage associated with them (Figure 5-3) that can vary from thin, diffuse layers around individual cells, colonies, or filaments to firm mucilaginous envelopes or sheaths (Komárek 2003a). In filamentous cyanobacteria with sheaths, the "filament" within the sheath is referred to as a trichome, and the term *filament* refers

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Figure 5-1 Some cyanobacteria species commonly form surface blooms

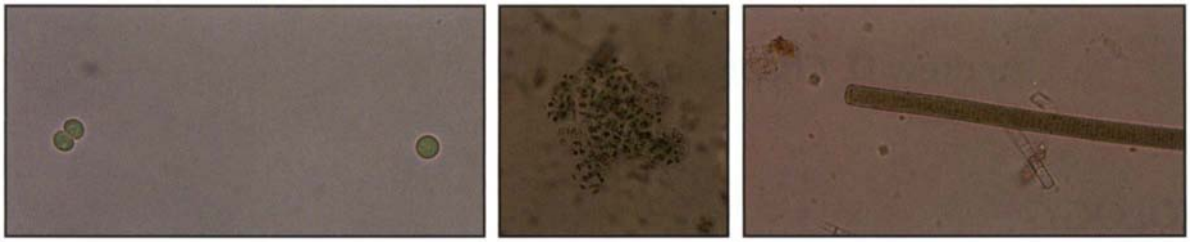

Figure 5-2 Cyanobacteria are morphologically diverse ranging from (a) unicellular to (b) colonial to (c) filamentous

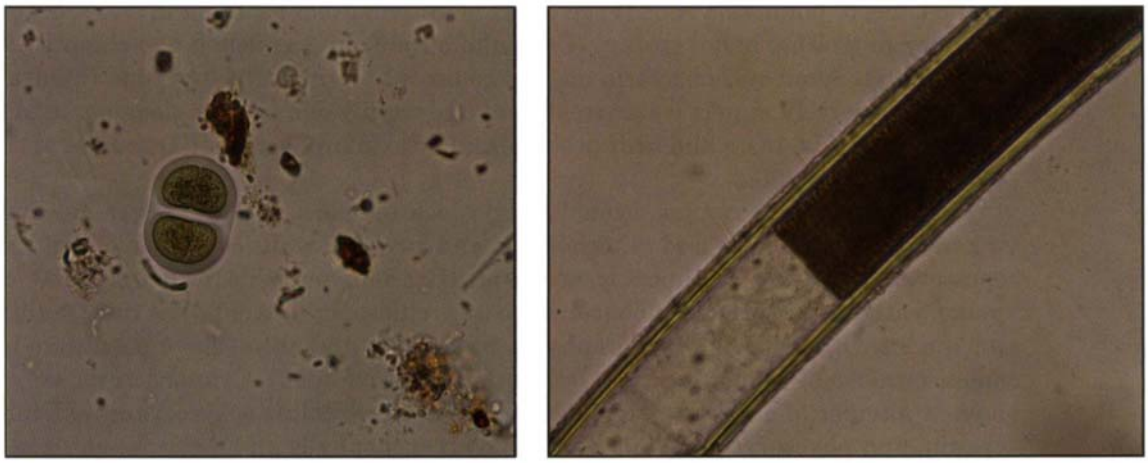

Figure 5-3 Many cyanobacteria have mucilage associated with them

to the trichome and its surrounding sheath. In filamentous forms lacking sheaths, the terms *filament* and *trichome* can be used interchangeably.

Cyanobacteria divide mainly by simple binary fission either by constriction (pinching) or septum formation followed by cleavage (Komárek 2003a). Cell division generally occurs in one, two, or three planes, which are more or less perpendicular to one another in successive generations (Komárek 2003a). In filamentous forms, cell division is typically perpendicular to the trichome axis (Komárek 2003a). Reproduction in unicellular cyanobacteria is through ordinary cell division and in colonial forms by liberation of single cells or clusters of cells from colonies, colony fragmentation, or production of

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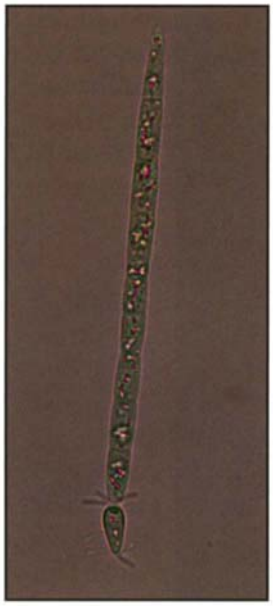

Figure 5-4 Aerotopes (gas vacuoles) allow some cyanobacteria to control their buoyancy

Figure 5-5 Heterocytes (heterocysts) are specialized cells found in some filamentous cyanobacteria where nitrogen fixation occurs

specialized reproductive cells (exocytes, nanocytes, and baeocytes) (Komárek 2003a). Reproduction in filamentous forms is mainly via fragmentation of portions of the filament into short, often motile, fragments called hormogonia (Komárek 2003a).

A number of specialized structures are found within the cyanobacteria, the most important in terms of their ecology and taxonomy being aerotopes, heterocytes, and akinetes. Aerotopes (gas vacuoles) are composed of groups of gas vesicles (Figure 5-4). Gas vesicles are hollow structures with walls that allow passage of gases but not water (Oliver and Ganf 2000). When a sufficient number of aerotopes are produced, cells, colonies, or filaments are able to float. Gas vesicles fill or collapse in response to changes in light level and the resultant changes in rates of photosynthesis and respiration that affect internal turgor pressure (Oliver and Ganf 2000). Aerotope production is highest at low light levels. Most of the well-known surface bloom–forming cyanobacteria (i.e., *Anabaena*, *Aphanizomenon,* and *Microcystis*) can produce aerotopes. Surface scums are most likely to form under calm conditions when cyanobacteria lose the ability to regulate their buoyancy (Walsby 1994). Some bloom-forming cyanobacteria with aerotopes, such as *Cylindrospermopsis* and *Planktothrix*, do not generally form surface scums.

Heterocytes (heterocysts) (Figure 5-5) are morphologically and physiologically distinct cells found in some cyanobacteria that allow atmospheric nitrogen (N_2) fixation (Paerl 1988). Heterocytes may be distinguished from vegetative cells by their yellow-hued transparent appearance and thick cell walls (Paerl 1988). Heterocytes provide an anaerobic environment in which nitrogen fixation can occur via the enzyme complex nitrogenase (Komárek 2003b). Some cyanobacteria that do not produce heterocytes are capable of nitrogen fixation but only under anoxic conditions or in microanaerobic zones commonly found in microbial mats (Stal 2000).

Akinetes (Figure 5-6) are specialized cells found in some filamentous cyanobacteria that may act as resting stages to survive adverse conditions (Komárek 2003b). Akinetes are distinguished from vegetative cells and heterocytes by their larger size, thick cell walls, and granular appearance (Paerl 1988). When exposed to favorable

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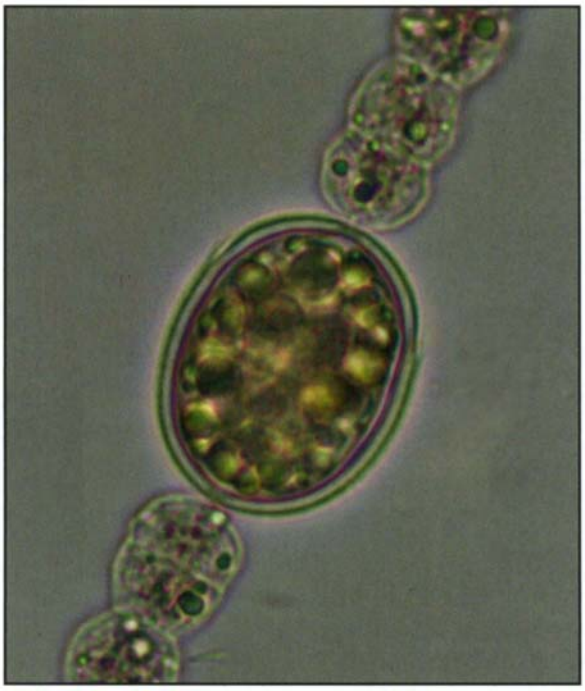

Figure 5-6 Akinetes are specialized resting cells found in some filamentous cyanobacteria

conditions for growth, akinetes germinate and new filaments begin to grow (Rother and Fay 1979, van Dok and Hart 1997).

TAXONOMY

Cyanobacteria taxonomy has a convoluted history. Traditionally, cyanobacteria have been classified following the International Code of Botanical Nomenclature with their taxonomy based primarily on microscopic and cytological characteristics (Anagnostidis and Komárek 1985). Once the prokaryotic nature of cyanobacteria was discovered it was suggested that cyanobacteria taxonomy follow the International Code of Nomenclature of Bacteria (Stanier et al. 1978). The bacteriological approach is based on genetic, morphological, physiological, cytological, and biochemical characteristics of species in axenic cultures (Anagnostidis and Komárek 1985). The most recent revision of cyanobacteria taxonomy (still in progress) uses a botanical classification scheme based on molecular data supplemented by all other types of available data including morphological diversity and variation, ecology, and ultrastructure (Komárek 2005). This approach is known as the "polyphasic approach" to cyanobacteria taxonomy (Komárek 2005).

Traditional cyanobacteria taxonomy splits the cyanobacteria into four orders easily recognized by morphological characteristics. The Chroococcales are unicellular and colonial (coccoid) forms; the Oscillatoriales are filamentous forms lacking true branching, heterocytes, and akinetes; the Nostocales are filamentous forms lacking true branching but with facultative production of heterocytes and akinetes; and the Stigonematales are filamentous forms with true branching (a result of cell division in multiple planes) and facultative production of heterocytes.

Recent ultrastructural (thylakoid arrangement) and molecular data indicate that the split of coccoid ("Chroococcales") and simple filamentous forms ("Oscillatoriales") is not a valid distinction at the highest level (Hoffmann et al. 2005) and that all forms with heterocytes ("Nostocales" and "Stigonematales") should be united (Gugger and

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Hoffmann 2004, Henson et al. 2004). Other important results of recent polyphasic studies are that the identification of *Microcystis* species using traditional morphological traits is not valid (Otsuka et al. 1999, 2001) and that the separation of *Anabaena* and *Aphanizomenon* is not supported by molecular data (Gugger et al. 2002, Rajaniemi et al. 2005). As yet, these findings have not been incorporated in any widely available monographs on cyanobacteria identification.

ECOLOGY

Cyanobacteria are commonly found in surface waters used as sources for drinking water. The location of cyanobacteria in the water column, their seasonality, and the species composition of the cyanobacteria community are all factors to be considered when managing a water body as a drinking water source. Different cyanobacteria species may utilize different habitats within a body of water. Planktonic forms may be found at or near the water surface (*Anabaena, Microcystis*), in subsurface layers (*Planktothrix rubescens*), or distributed throughout the mixed zone (*Cylindrospermopsis raciborskii, Planktothrix agardhii*). Benthic forms (i.e., *Oscillatoria, Phormidium,* and *Lyngbya*) can be found where sufficient light reaches the bottom, forming loose mats at the sediment surface or attached to firm substrates such as rock. Periphytic cyanobacteria can be found attached to a variety of substrates including emergent, floating, or submerged vegetation, submerged woody debris, human-made structures such as walls and pipes, and even other algae.

Seasonal patterns of cyanobacteria can be affected by a number of interacting variables including geography, lake/reservoir morphometry, nutrient availability, light availability, weather patterns, competition with other algae, and grazing. In temperate waters, cyanobacteria generally exhibit peak abundance in the late summer months, whereas in tropical and subtropical regions, cyanobacteria can dominate at any time of the year and perhaps on a permanent basis. Lake morphometry in conjuction with seasonal changes in temperature and irradiance affects cyanobacteria seasonality by dictating the mixing pattern of a water body. In water bodies that exhibit seasonal stratification, cyanobacteria generally are most abundant after stratification has been in place for a prolonged period.

Cyanobacteria are most common in alkaline, nutrient-rich waters. Phosphorus and nitrogen are the nutrients most often associated with limiting growth of cyanobacteria and other algae. Because phosphorus requirements can vary widely between groups and species of algae, phosphorus availability and limitation can play an important role in competitive interactions (Huisman and Hulot 2005). Many cyanobacteria are poor competitors for phosphorus compared to diatoms and green algae (Tilman et al. 1986). Like most other groups of algae, cyanobacteria are capable of luxury uptake and storage of phosphorus (as polyphosphates) when it is available in surplus. This ability is particularly advantageous to those species, such as *Microcystis*, that are able to regulate their buoyancy and so move down to deeper, phosphorus-rich waters when surface phosphorus levels become depleted (Ganf and Oliver 1982).

Ammonia is the preferred nitrogen source of cyanobacteria, though nitrate, nitrite, and N_2 (in nitrogen-fixing species) can also be assimilated (Oliver and Ganf 2000). Nitrogen-fixing cyanobacteria have a major competitive advantage relative to other cyanobacteria and algae when other sources of inorganic nitrogen are low (Oliver and Ganf 2000). Nitrogen fixation is physiologically expensive and is only triggered when available inorganic nitrogen drops below a critical level (Horne and Commins 1987). Unlike other groups of algae, cyanobacteria are capable of nitrogen storage in the form of cyanophycin granules and in the pigment phycocyanin (Oliver and Ganf 2000).

As photosynthetic organisms, both light intensity and quality (wavelength; i.e., blue, red, green) are important factors in growth of cyanobacteria. In some systems,

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such as those with high levels of tannins ("blackwater"), light availability can be the primary factor limiting growth of cyanobacteria and other algae (Aldridge et al. 1998). Both the quantity and quality of light (photosynthetically active radiation or PAR) change with depth in the water column. These changes are due to light attenuation and absorption by water itself and that caused by dissolved organic matter, inorganic compounds, and algae suspended in the water (Kirk 1983). As a result of changes in light climate with depth, cyanobacteria moving through the water column, either due to mixing or vertical migration, will encounter changes in PAR intensity and spectral distribution (Oliver and Ganf 2000). At high light intensities, photoinhibition of photosynthesis may occur, while at low light levels, the critical light threshold required for photosynthesis may not be reached. As in other groups of algae, there are light requirement differences between species (Foy et al. 1976), with some cyanobacteria preferring higher light environments (*Microcystis aeruginosa*) and some lower (*Limnothrix redekei*). Cyanobacteria can adapt to changes in light intensity and quality by changing the size and number of photosynthetic units and changing the ratio of chl-*a* to accessory pigments (Oliver and Ganf 2000).

Weather patterns, both short-term (Soranno 1997) and long-term (Bouvy et al. 2000), can affect cyanobacteria abundance and dominance. Rainfall and associated runoff can affect nutrient availability. Blooms often occur when heavy rains, resulting in high nutrient runoff, are followed by a prolonged dry, calm period. Blooms themselves often crash after heavy storms occur. Strong winds may terminate blooms by redistributing cells throughout the water column and exposing them to unfavorable light conditions, while lighter winds may concentrate surface scums along shorelines. Periods of drought may lead to low flow in reservoirs, with longer retention times playing a role in increased buildup of nuisance cyanobacteria populations (Bouvy et al. 2000, Fabbro and Duivenvoorden 2000). There is some concern that global warming may lead to warmer water temperatures and other conditions that would favor development of cyanobacteria blooms (Padisak 1998, Briand et al. 2004).

Competition between algal species occurs along changing gradients of critical resources. Every algal species has optima for each of the various environmental conditions (i.e., light, water temperature, pH, nutrients) controlling growth and ultimate success or failure in the phytoplankton community. If a species is a better competitor for the resource limiting growth, then it will continue to grow at the expense of other species. Blooms result when conditions favoring growth of a given species remain in place long enough for a large biomass to accumulate. For cyanobacteria blooms, those conditions generally include a stable water column (Rolland et al. 2005). Water column stability favors species, such as cyanobacteria with gas vesicles, which are able to control their vertical position. Such species have several advantages over sinking forms. They can move to nutrient-rich lower waters at night, then move to surface waters during the day where their optimum light level can be obtained; they can accumulate at the surface, thereby shading out potential competitors while at the same time utilizing atmospheric sources of CO_2 (Paerl 1988); or they can accumulate at some depth below the surface where conditions are most favorable (Dokuli and Teubner 2000).

Grazing of algae by zooplankton can have a major impact on phytoplankton community structure. Many zooplankton preferentially graze on small algal cells (<20 μm) and can remove substantial quantities of such phytoplankton species from the water. The removal of small phytoplankton species may favor dominance by larger, less edible forms of algae such as filamentous and colonial cyanobacteria. Not only are large cyanobacteria mechanically difficult for many zooplankton to ingest (Gliwicz and Lampert 1990), they are also of relatively low nutritional value compared to other algal groups, many appear to be unpalatable, and some may be toxic (Lampert 1981, Gilbert 1994, Ferrão-Filho et al. 2000, Visser et al. 2005). Cyanobacteria have also

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been observed passing live through the digestive tract of some zooplankton species (de Infante 1978). Rotifers and protozoans appear to be more successful (Paerl 1988) in the grazing of cyanobacteria than crustacean zooplankton (cladocerans and copepods).

A wide range of cyanobacteria may be found in lakes and reservoirs used as source water. These include small (<2 μm) unicellular picocyanobacteria, microscopic colonial and filamentous forms, and macroscopic colonies and filament aggregates. Of particular concern to water treatment facilities are those cyanobacteria species capable of producing blooms, taste and odor compounds, or toxins. Many bloom-forming cyanobacteria are also known taste and odor and/or toxin producers. The majority of bloom-forming species are in the genera *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Microcystis*, *Planktothrix*, and *Woronichinia*.

SIGNIFICANCE

Aside from being aesthetically unpleasant, cyanobacteria blooms can have a number of other adverse impacts on water quality, including reduced transparency that may negatively impact submersed plant beds, impaired food web dynamics, decreased biodiversity, and potential oxygen depletion that may result in fish kills (Dokuli and Teubner 2000). Cyanobacteria blooms in source water may result in filter clogging, which reduces the length of filter runs and leads to frequent backwashings (AWWA 2002).

Another major potential issue with the presence of cyanobacteria in source water is the production by some species of potent taste and odor compounds (Watson 2003). Some of these metabolites may be detected in drinking water at levels of ≤10 ng/mL (AWWA 2002). While there is no evidence for taste and odor compounds having adverse health effects on humans, their presence in drinking water leads to customer complaints and undermines consumer confidence in the safety of a water supply (McGuire 1995, Jardine et al. 1999).

The two most common taste and odor compounds produced by cyanobacteria are geosmin and MIB (2-methylisoborneol), which impart a musty-earthy taste and odor to the water. Geosmin is primarily made by species in the Nostocales and Oscillatoriales, including the well-known bloom formers *Anabaena circinalis*, *Aphanizomenon flos-aquae*, and *P. agardhii* (Watson 2003). MIB is produced by some members of the Oscillatoriales, including some species of *Oscillatoria*, *Phormidium*, and *Pseudanabaena* (Watson 2003). A number of other taste and odor compounds may be released by cyanobacteria including a variety of organic sulfur compounds by species of *Microcystis* (Watson 2003).

Taste and odor compounds can be produced by both planktonic and benthic species. This can be important with regard to best location and depth for water intake. Lack of an obvious "waterbloom" does not guarantee that taste and odor problems will not occur, for example, if benthic mats of *Phormidium* or *Pseudanabaena* are present.

Negative impacts due to cyanobacteria taste and odor production include the aforementioned public perception and the cost of removal of the offending compounds. Taste and odor metabolites are difficult to remove except with ozone or granular activated carbon (AWWA 2002). Some taste and odor compounds can impart off-flavor to fish, which may be an issue on multiuse water bodies where fishing occurs.

In addition to taste and odor compounds, a number of cyanobacteria produce metabolites (cyanotoxins) that may be harmful to aquatic and terrestrial organisms (Mazur-Marzec 2006). Intermittent but widespread cases of cyanotoxin poisoning of wild and domestic animals have been documented (Carmichael et al. 2001). Verified cases of cyanotoxin poisoning in humans are uncommon, but incidents have occurred throughout the world (Falconer 2005). Human cyanotoxin poisonings may be rare primarily because people generally avoid direct contact with surface scums, and drinking

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water treatment processes usually prevent high concentrations of toxin in finished water (Carmichael et al. 2001). The primary routes of exposure are through oral consumption (via drinking water or ingestion during recreation), inhalation, and skin absorption (Falconer 2001). Effects can be acute (gastrointestinal distress up to death depending on exposure) or chronic (tumor promotion) (Falconer and Humpage 1996).

Most cyanotoxins belong to three major classes: cyclic peptides, alkaloids, and endotoxins (Carmichael 1997, Codd et al. 2005, Falconer 2005). Cyclic peptides include the hepatotoxins ("liver toxins") microcystin and nodularin. The former is produced by species belonging to a wide range of genera including *Anabaena, Limnothrix, Microcystis, Oscillatoria,* and *Planktothrix,* and the latter only by the brackish water species *Nodularia spumigena* (Cronberg et al. 2003). Microcystin is the most commonly reported cyanotoxin worldwide (Carmichael 2001).

There are three types of alkaloids: neurotoxic, cytotoxic, and dermatotoxic. Neurotoxic alkaloids include the neurotoxins anatoxin-a, anatoxin-a(s), homoanatoxin-a, saxitoxin, and neosaxitoxin. The primary targets of these toxins are nerve axons and synapses. Some common producers of anatoxin include multiple species of *Anabaena, Aphanizomenon,* and *Planktothrix* (Cronberg et al. 2003). Common saxitoxin producers include *A. circinalis,* several *Aphanizomenon* species, *C. raciborskii,* and *Lyngbya wollei* (Cronberg et al. 2003).

Cylindrospermopsin is a cytotoxic alkaloid that primarily affects the liver and kidneys. Originally described from *C. raciborskii,* it has since been found in a number of other taxa including *Anabaena bergii* and *Aphanizomenon ovalisporum* (Cronberg et al. 2003) and most recently in *Aph. flos-aquae* (Preussel et al. 2006) and *L. wollei* (Seifert et al. 2007).

Dermatotoxic alkaloids include the aplysiatoxins and lyngbyatoxins, whose primary target is the skin. These compounds are produced by some species in the filamentous genera *Lyngbya* and *Phormidium*. Contact with mats of dermatoxic alkaloids producing cyanobacteria sometimes results in painful rashes.

Endotoxins include lipopolysaccharides that are found in outer membranes of Gram-negative bacteria and cyanobacteria. These lipopolysaccharides can cause gastrointestinal distress and elicit irritant and allergenic responses in some people and animals.

Recently it has been reported that the nonprotein amino acid β-*N*-methylamino-L-alanine (BMAA) has neurotoxic properties (Cox et al. 2005). BMAA, originally found in an endosymbiotic species of *Nostoc,* was shown to be produced by some species in all cyanobacteria groups (Cox et al. 2005).

MANAGEMENT AND MITIGATION OF CYANOBACTERIA AND THEIR TOXINS

Management of cyanobacterial harmful algal blooms has two main components: mitigation and prevention (Piehler 2008). Mitigation involves control/removal of cyanobacteria and their toxins once they are present in a system. This can include application of algicides (such as copper sulfate), oxidants, and coagulants (Hrudey et al. 1999); physical removal of cyanobacteria scums or mats; and the use of mechanical mixing to create unstable conditions in the water column. Application of algicides needs to occur when potentially toxic species are at relatively low densities so that large releases of toxin into the water source do not occur. All of these mitigation methods are most effective in small water bodies.

At both large and small spatial scales, the most effective long-term solution for controlling cyanobacterial blooms is to prevent the environmental conditions that favor their development. The most common strategies used are controlling nutrient inputs

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into the system and manipulating water residence time (Falconer 2005, Piehler 2008). For nutrient control to be effective, the limiting nutrient(s) must be determined, the sources of these nutrients identified, and nutrient reduction goals set. Manipulation of flow and flushing rates in managed systems has been shown to be effective in controlling cyanobacterial populations by physically removing cyanobacteria and reducing stratification (Maier et al. 2001, Falconer 2005).

Monitoring of source water for potentially toxigenic cyanobacteria is critical if the source is a productive system prone to cyanobacteria blooms. Early detection of toxin-producing cyanobacteria allows for a proactive response rather than a reactive one when a bloom has already formed and may potentially provide a large cost savings by avoiding the expensive treatment options necessary under bloom conditions.

Conventional water treatment processes (coagulation/flocculation, sedimentation, and rapid sand filtration) may not be effective for cyanotoxin removal, particularly if prechlorination is used, as this lyses the cyanobacteria cells, thus releasing the intracellular toxins into the water (Newcombe 2002, Falconer 2005). At present, the most effective removal of cyanotoxins from drinking water is obtained through postclarification ozonation followed by granulated activated carbon filtration (Falconer 2005). However, such treatment is expensive, and if cyanobacteria blooms are only a seasonal problem, more cost-effective treatment options, such as the addition of powdered activated carbon when necessary, may suffice (Falconer 2005). Postchlorination may eliminate microcystin and cylindrospermopsin, making it through the treatment process but only if a residual chlorine level of at least 0.5 mg/L is maintained for more than 30 minutes in the finished water at neutral pH (Falconer 2005).

The World Health Organization (WHO) in 1998 established a provisional guideline for microcystin-LR in drinking water of 1.0 μg/L. Since that time, a number of countries have adapted the WHO microcystin guideline, including Brazil, Germany, Poland, Czech Republic, France, Spain, and New Zealand (Chorus 2005). Australia has set a microcystin guideline of 1.3 μg/L and Canada, 1.5 μg/L. Australia is also considering a 3-μg/L limit for anatoxin-a and saxitoxin and a range of 1–15 μg/L for cylindrospermopsin. Brazil is recommending guidelines of 3 μg/L for saxitoxins and 15 μg/L for cylindrospermopsin. The United States has no established guidelines for any cyanotoxins at this time, although cyanobacteria and cyanotoxins have been added to the US Environmental Protection Agency's Contaminant Candidate List (CCL 1 and 2) with microcystins, cylindrospermopsin, and anatoxin-a given highest priority.

IDENTIFICATION

Generally, for those charged with recognition of cyanobacteria in source waters, identifications will likely be based on morphological characteristics observed under a light microscope. The first step in this process is to determine to what genus a species of interest belongs. Important morphological traits used to identify cyanobacteria vary depending on thallus structure (unicell, colony, or filament). In unicells, the shape of the cells and appearance of the cell contents are the main traits used to differentiate between genera. In colonial forms, cell shape, cell arrangement, nature of the mucilage, colony shape, and presence/absence of aerotopes are the main features used to distinguish between genera. For filamentous forms, ability to produce heterocytes and/or akinetes, trichome width, cell shape, presence/absence and nature of sheaths, trichome morphology, presence/absence and nature of branching, presence/absence of aerotopes, and location of heterocytes and akinetes on trichome are all important traits used to differentiate between genera.

Because not all species in a genus may produce taste and odor compounds and/or toxins, identification to the species level is often needed to make fully informed management decisions. Identifying a potential nuisance cyanobacteria to species allows

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for a more cost-effective analysis by narrowing down the compounds to analyze for. Species identification can be extremely difficult and in some cases may be impossible if certain critical features (e.g., akinetes) are not present.

Once an unknown cyanobacteria has been identified to the genus, a number of traits may be used in an attempt at species identification. For colonial species, these include but are not limited to mucilage characteristics, colony shape, cell size and shape, presence/absence of aerotopes, and color. For filamentous forms, some useful traits for distinguishing between species include trichome morphology, presence/absence and nature of sheath, presence/absence of constrictions at cross-walls, presence/absence and type of branching, end cell shape, motility, shape, size and location of heterocytes and akinetes, cell size and shape, color, and granulation. While some cyanobacteria species have a worldwide distribution (i.e., are cosmopolitan), many others are restricted to specific geographic ranges or habitats.

Molecular methods are also used for cyanobacteria identification; see Section I, Chapter 2, for more information.

KEY TO COMMON CYANOBACTERIA GENERA

^{TO} = One or more species in genus known or suspected to produce taste/odor compounds

^{TX} = One or more species in genus known or suspected to produce toxins

1a. Cells solitary or grouped in irregular clusters, not forming mucilaginous colonies; cells ovate or cylindrical, *Synechococcus*^{TO,TX} (Figure 5-7)

1b. Cells organized in mucilaginous colonies or in trichomes, 2

2a. Cells in few to many celled colonies, 3

2b. Cells arranged in trichomes (filaments), 16

3a. Colonies spherical to irregularly reticulate; cells in rows, embedded in mucilaginous strands (difficult to see in older colonies), *Cyanodictyon* (Figure 5-8)

3b. Cells not embedded in mucilaginous strands, 4

4a. Colonies ± spherical, cells clustered in colony center with short rows of cells radiating in all directions toward the cell periphery, *Radiocystis* (Figure 5-9)

4b. Colonies without rows of cells radiating from center of colony, 5

5a. Colonies cubic; cells arranged three-dimensionally in perpendicular rows, *Eucapsis* (Figure 5-10)

5b. Colonies not cubic, 6

6a. Cells regularly arranged and forming flat (mostly), rectangular plates, *Merismopedia* (Figure 5-11)

6b. Cells forming spherical, ovate, or irregular colonies, 7

7a. Colony hollow with cells arranged toward periphery or in one layer just beneath the mucilage surface, 8

7b. Cells distributed throughout colonial mucilage; colony not hollow, 12

8a. Cells attached to thin or thick stalks radiating from colony center (staining is often required to see stalks clearly), 9

8b. Cells not attached to radial stalks, 11

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9a. Cells attached to thin, threadlike stalks; cells generally not densely distributed, *Snowella*[TX] (Figure 5-12)

9b. Cells attached to wide, mucilaginous stalks; cells more densely packed, 10

10a. Cells spherical, oval, or obovoid; cells up to 6 μm in length, *Woronichinia*[TX] (Figure 5-13)

10b. Cells obovoid, club-shaped, or cordiform; cells usually over 6 μm in length, *Gomphosphaeria* (Figure 5-14)

11a. Cells spherical, arranged in one, sometimes two, layers on the periphery, *Coelosphaerium*[TX] (Figure 5-15)

11b. Cells slightly elongate or obovoid, *Coelomoron* (Figure 5-16)

12a. Colonies usually few celled (2–16 cells); mature cells in most species hemispherical in shape, *Chroococcus* (Figure 5-17)

12b. Colonies many celled; mature cells not hemispherical, 13

13a. Cells elongate, widely oval to rod-shaped, *Aphanothece* (Figure 5-18)

13b. Cells spherical to slightly ovate, 14

14a. Individual cells and groups of cells surrounded by wide, concentrically lamellate mucilaginous envelopes, *Gloeocapsa* (Figure 5-19)

14b. Individual cells and groups of cells not surrounded by wide, concentrically lamellate mucilaginous envelopes, 15

15a. Cells without aerotopes, *Aphanocapsa* (Figure 5-20)

15b. Cells with aerotopes, *Microcystis*[TO,TX] (Figure 5-21)

16a. Trichomes with heterocytes, 17

16b. Trichomes without heterocytes, 23

17a. Mature heterocytes at end(s) of trichome (terminal), 18

17b. Mature heterocytes not at end(s) of trichome (intercalary), 20

18a. Forms hemispherical or globose colonies of radiating trichomes; trichomes with heterocyte at one end and sharply attenuated at the other, *Gloeotrichia*[TX] (Figure 5-22)

18b. Does not form colonies of radiating trichomes, 19

19a. Mature heterocytes at both ends of trichome; heterocytes spherical, ovate, or ellipsoid in shape; development of heterocytes intercalary, *Anabaenopsis*[TX] (Figure 5-23)

19b. Heterocytes at one or both ends of trichome (but often absent); mostly teardrop shaped; heterocyte development terminal, *Cylindrospermopsis*[TX] (Figure 5-24)

20a. Cells compressed, disc shaped, sheath generally visible, *Nodularia*[TX] (Figure 5-25)

20b. Cells spherical, barrel shaped, or cylindrical, 21

21a. Forms clumps of tangled trichomes surrounded by copious, thick mucilage, *Nostoc*[TX] (Figure 5-26)

21b. Copious, thick mucilage not present, 22

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22a. Cells generally spherical or barrel shaped, end cells not elongated; heterocytes evenly distributed along trichome, *Anabaena*[TO,TX] (Figure 5-27)

22b. Cells generally cylindrical, end cells often elongated and attenuated; heterocytes not evenly distributed along trichome, *Aphanizomenon*[TO,TX] (Figure 5-28)

23a. Trichomes with sheaths, 24

23b. Trichomes lacking sheaths, 26

24a. Filaments thin (<3 μm wide), *Planktolyngbya* (Figure 5-29)

24b. Filaments wider (>3 μm wide), 25

25a. Cells discoid, *Lyngbya*[TO,TX] (Figure 5-30)

25b. Cells longer than wide, isodiametric or shorter than long (not discoid), *Phormidium*[TO,TX] (in part) (Figure 5-31)

26a. Trichomes spirally twisted, *Arthrospira*[TX]/*Spirulina* (Figures 5-32, 5-33)

26b. Trichomes not spirally twisted, 27

27a. Cells length always less than one-half the cell width, *Oscillatoria*[TO,TX] (Figure 5-34)

27b. Cells length always greater than one-half the cell width than broad, 28

28a. Trichomes <3 μm wide, 29

28b. Trichomes >3 μm wide, 30

29a. Cells strongly constricted; sometimes appearing disconnected; aerotopes mostly absent, *Pseudanabaena*[TO,TX] (Figure 5-35)

29b. Cells not or only weakly constricted at the cross walls; localized aerotopes, *Limnothrix*[TX] (Figure 5-36)

30a. Cells with aerotopes, *Planktothrix*[TO,TX] (Figure 5-37)

30b. Cells lacking aerotopes, *Phormidium*[TO,TX] (in part) (Figure 5-38)

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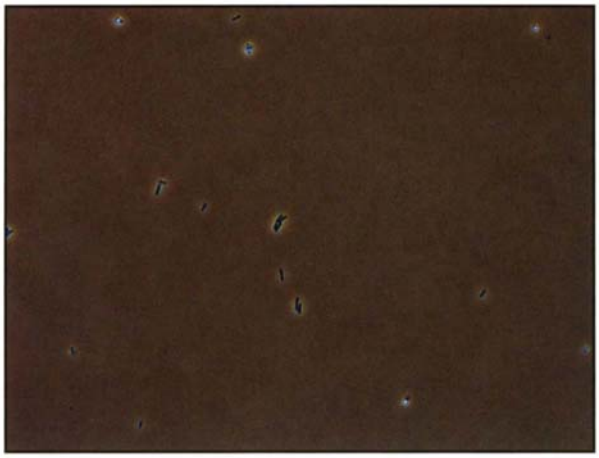

Figure 5-7 *Synechococcus*

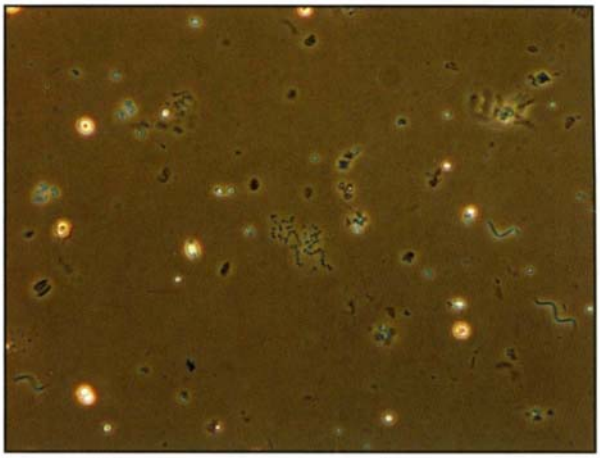

Figure 5-8 *Cyanodictyon*

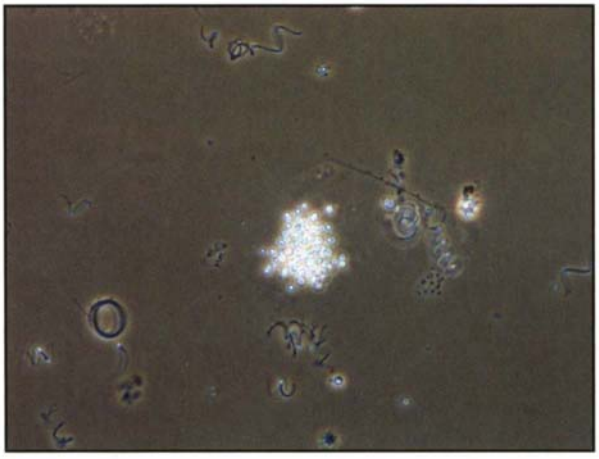

Figure 5-9 *Radiocystis*

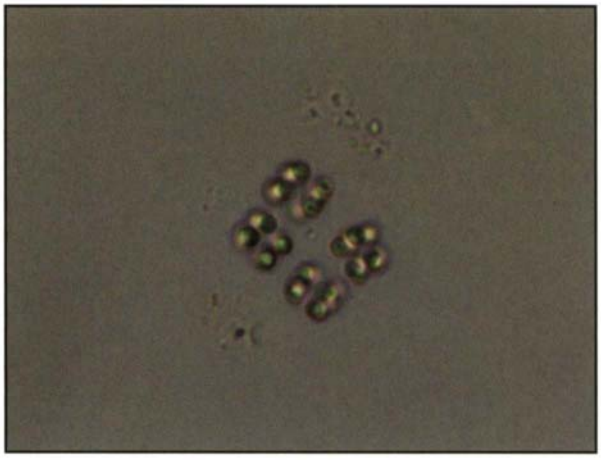

Figure 5-10 *Eucapsis*

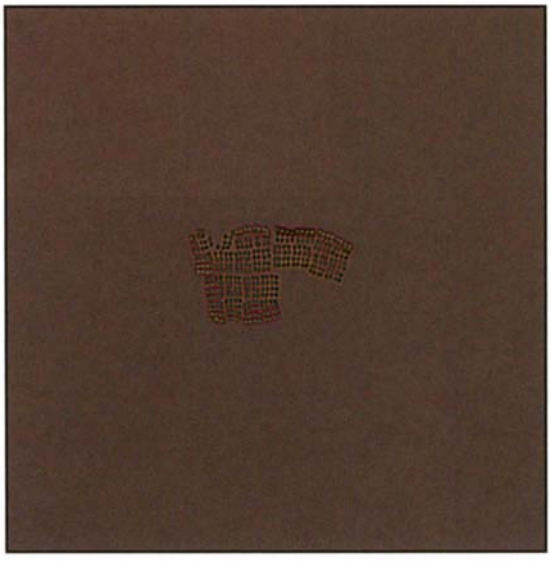

Figure 5-11 *Merismopedia*

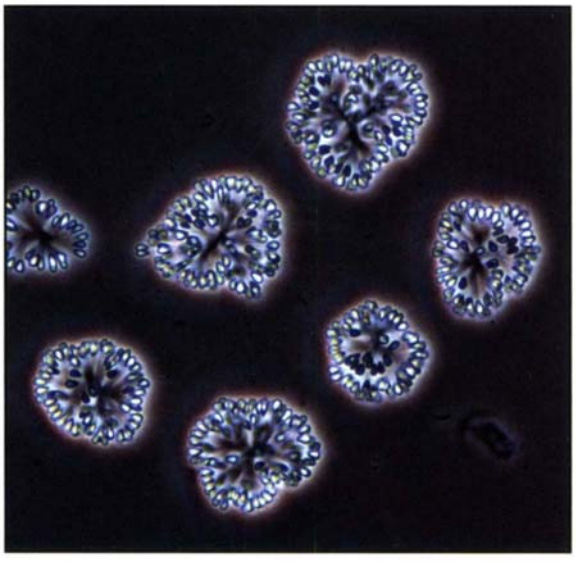

Figure 5-12 *Snowella* (Courtesy of Ann St. Amand, PhycoTech)

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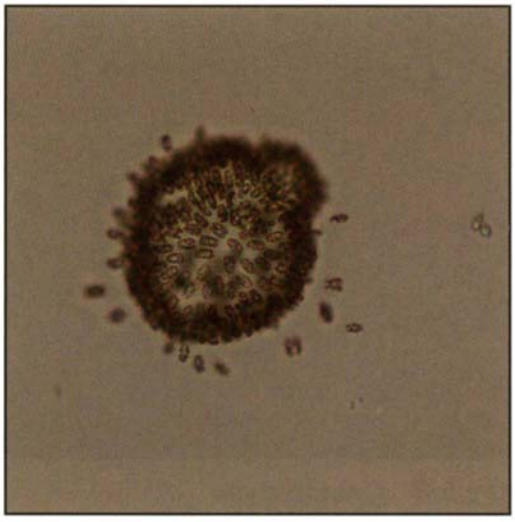

Figure 5-13 *Woronichinia*

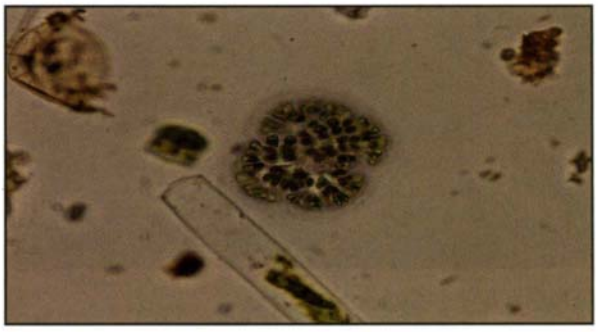

Figure 5-14 *Gomphosphaeria*

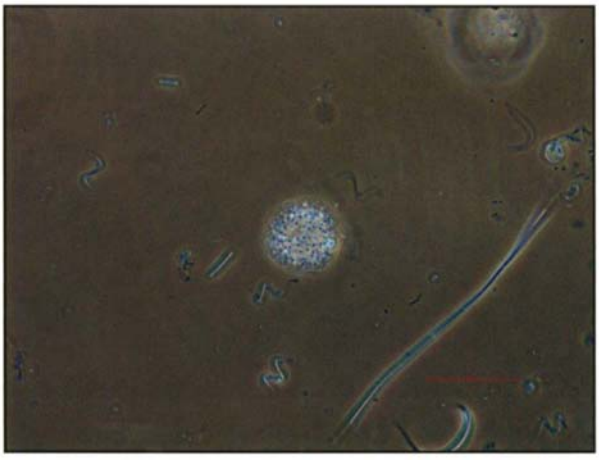

Figure 5-15 *Coelosphaerium*

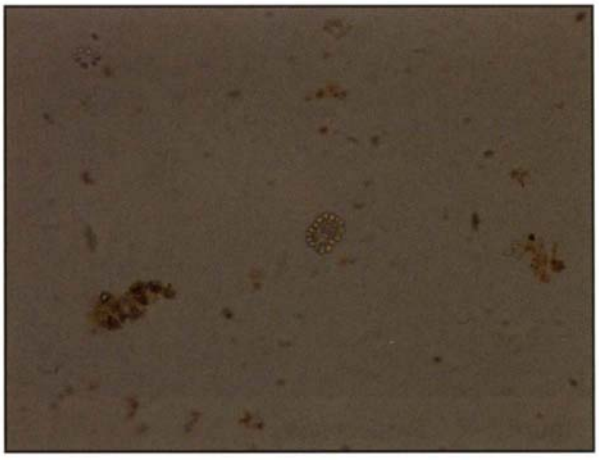

Figure 5-16 *Coelomoron*

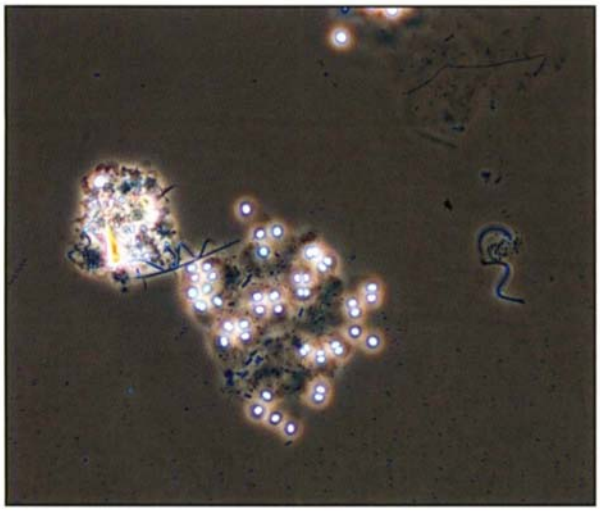

Figure 5-17 *Chroococcus*

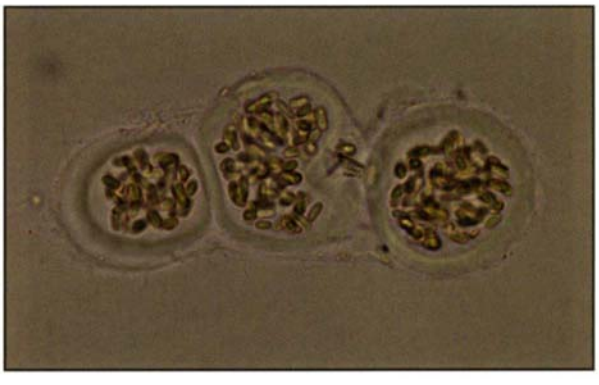

Figure 5-18 *Aphanothece*

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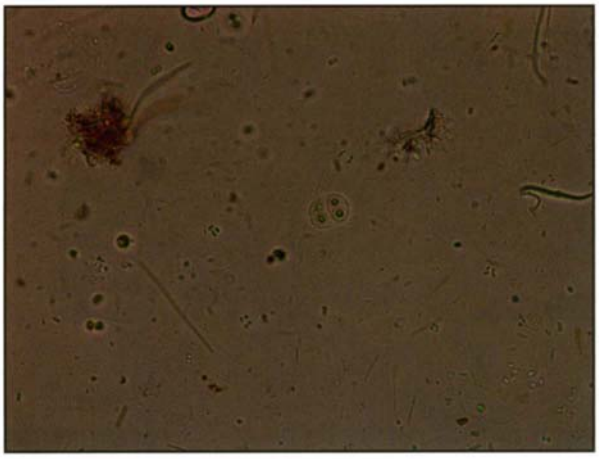

Figure 5-19 *Gloeocapsa*

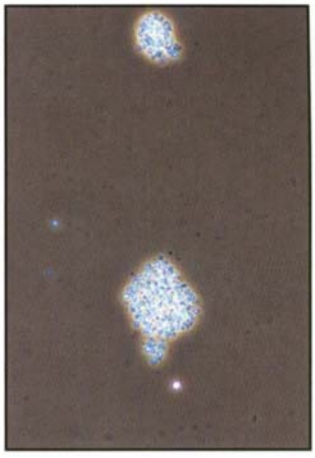

Figure 5-20 *Aphanocapsa*

Figure 5-21 *Microcystis*

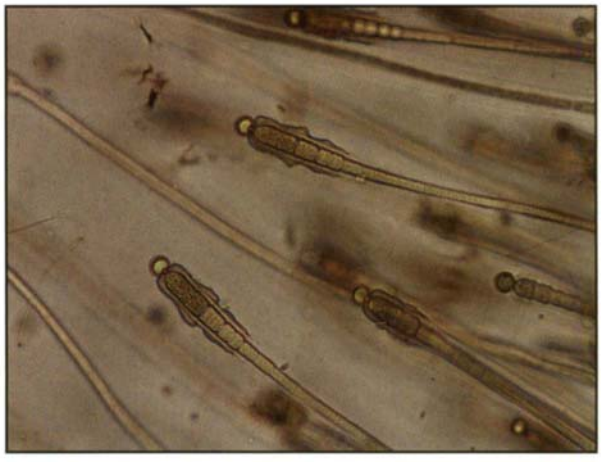

Figure 5-22 *Gloeotrichia*

Figure 5-23 *Anabaenopsis*

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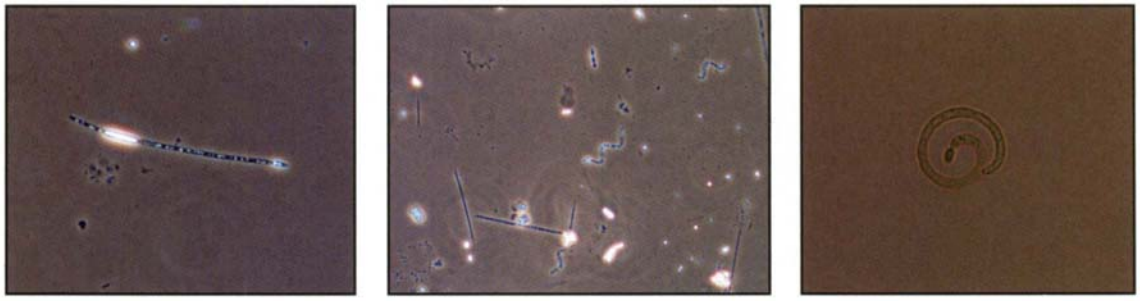

Figure 5-24 *Cylindrospermopsis*

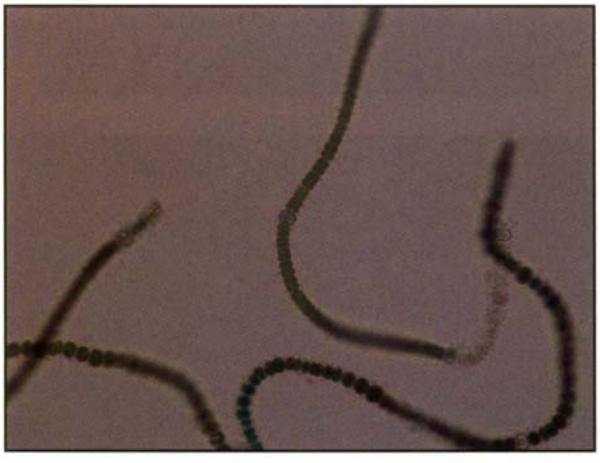

Figure 5-25 *Nodularia*

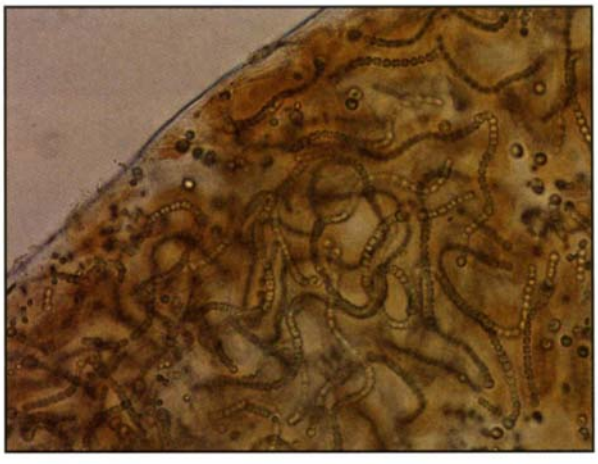

Figure 5-26 *Nostoc*

Figure 5-27 *Anabaena*

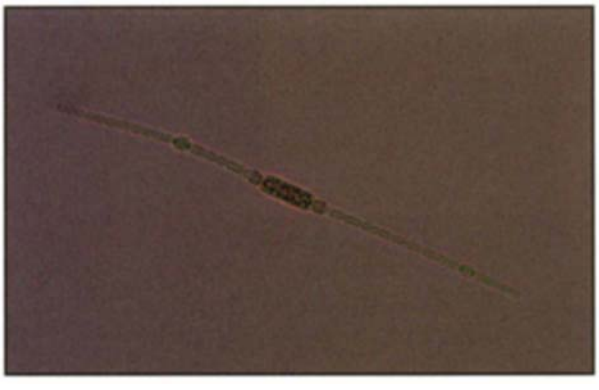

Figure 5-28 *Aphanizomenon*

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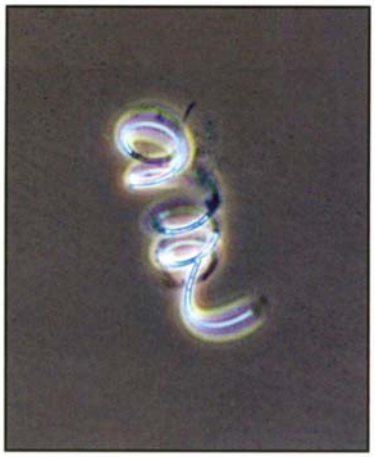

Figure 5-29 *Planktolyngbya*

Figure 5-30 *Lyngbya*

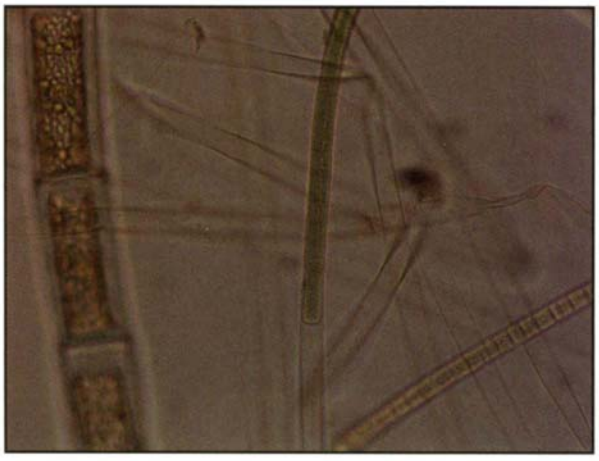

Figure 5-31 *Phormidium* (in part)

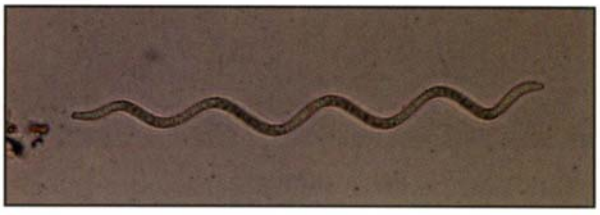

Figure 5-32 *Arthrospira*

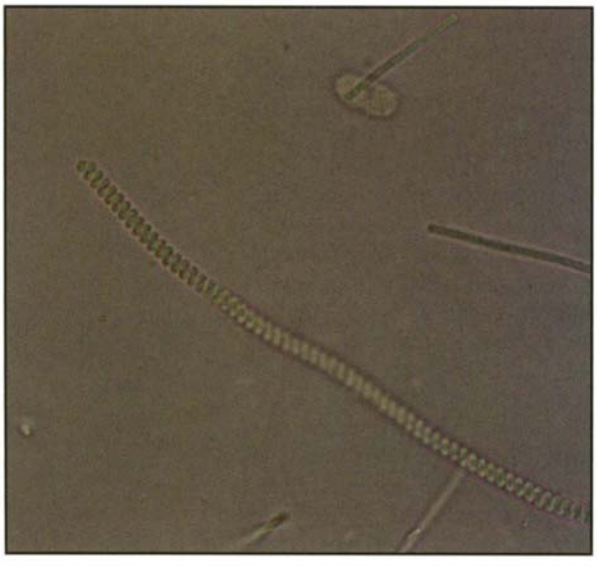

Figure 5-33 *Spirulina*

Figure 5-34 *Oscillatoria*

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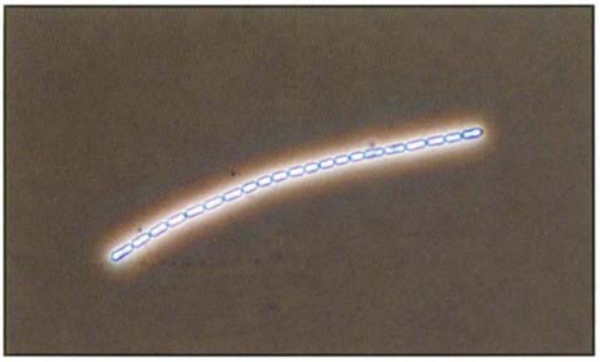

Figure 5-35 *Pseudanabaena*

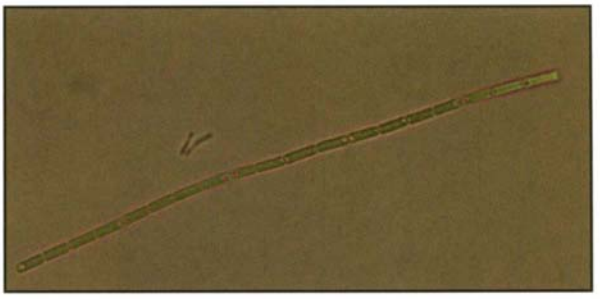

Figure 5-36 *Limnothrix*

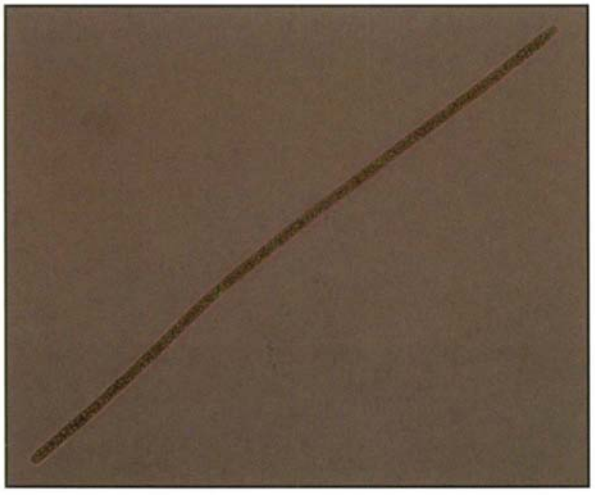

Figure 5-37 *Planktothrix*

Figure 5-38 *Phormidum* (in part)

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REFERENCES

Aldridge, F.J., A.D. Chapman, C.L. Schelske, and R.W. Brody. 1998. Interaction of light, nutrients and phytoplankton in a blackwater river, St. Johns River, Florida, USA. *Verh. Int. Verein. Limnol.*, 26:1665–1669.

American Water Works Association (AWWA). 2002. *Identification of algae in water supplies.* CD-ROM. Denver, Colo.: AWWA.

Anagnostidis, K., and J. Komárek. 1985. Modern approach to the classification system of cyanobacteria. 1. Introduction. *Algol. Stud.*, 38/39:291–302.

Bartram, J., W.W. Carmichael, I. Chorus, G. Jones, and O.M. Skulberg. 1999. Introduction. In *Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management.* I. Chorus and J. Bartram, eds. London: E&FN Spon.

Bouvy, M., D. Falcão, M. Marinho, M. Pagano, and A. Moura. 2000. Occurrence of *Cylindrospermopsis raciborskii* (cyanobacteria) in 39 Brazilian tropical reservoirs during the 1998 drought. *Aquat. Microb. Ecol.*, 23:13–27.

Briand, J.-F., C. Leboulanger, J.-F. Humbert, C. Bernard, and P. Dufour. 2004. *Cylindrospermopsis raciborskii* (cyanobacteria) invasion at mid-latitudes: Selection, wide physiological tolerance, or global warming? *Jour. Phycol.*, 40:231–238.

Brodo, I.M., S.D. Sharnoff, and S. Sharnoff. 2001. *Lichens of North America.* New Haven, Conn.: Yale University Press.

Carmichael, W. 1997. The cyanotoxins. *Adv. Botan. Res.*, 27:211–240.

Carmichael, W. 2001. *Assessment of blue-green algal toxins in raw and finished drinking water.* Denver, Colo.: Awwa Research Foundation.

Carmichael, W.W., M.F.O. Azevedo, J.S. An, R.J.R. Molica, E.M. Jochimsen, S. Lau, K.L. Rinehart, G.R. Shaw, and G.K. Eaglesham. 2001. Human fatalities from cyanobacteria: chemical and biological evidence for cyanotoxins. *Environ. Health Perspect.*, 109:663–668.

Castenholz, R.W. 1969. Thermophilic blue-green algae and the thermal environment. *Bact. Rev.*, 33:476–504.

Chorus, I. (ed.). 2005. *Current approaches to cyanotoxin risk assessment, risk management and regulation in different countries.* Berlin: Federal Environmental Agency.

Codd, G.A., J. Lindsay, F.M. Young, L.F. Morrison, and J.S. Metcalf. 2005. Harmful cyanobacteria: From mass mortalities to management measures. In *Harmful cyanobacteria.* J. Huisman, H.C.P. Mathis, and P.M. Visser, eds. New York: Springer.

Cox, P.A., S.A. Banack, S.J. Murch, U. Rasmussen, G. Tien, R.R. Bidigare, J.S. Metcalf, L.F. Morrison, G.A. Codd, and B. Bergman. 2005. Diverse taxa of cyanobacteria produce β-*N*-methylamino-L-alanine, a neurotoxin amino acid. *PNAS*, 102:5074–5078.

Cronberg, G., E.J. Carpenter, and W.W. Carmichael. 2003. Taxonomy of harmful cyanobacteria. In *Manual on harmful marine microalgae.* G.M. Hallegraeff, D.M. Anderson, and A.D. Cembella, eds. Paris: UNESCO Publishing.

de Infante, A. 1978. Natural food of the herbivorous zooplankton of Lake Valencia, (Venezuela). *Arch. Hydrobiol.*, 82:347–358.

Dokuli, M.T., and K. Teubner. 2000. Cyanobacterial dominance in lakes. *Hydrobiologia*, 438:1–12.

Fabbro, L.D., and L.J. Duivenvoorden. 2000. A two-part model linking multidimensional environmental gradients and seasonal succession of phytoplankton assemblages. *Hydrobiologia*, 438:13–24.

Falconer, I.R. 2001. Toxic cyanobacterial bloom problems in Australian waters: Risks and impacts on human health. *Phycologia*, 40:228–233.

Falconer, I.R. 2005. *Cyanobacterial toxins of drinking water supplies: Cylindrospermopsins and microcystins.* Boca Raton, Fla.: CRC Press.

Falconer, I.R., and A.R. Humpage. 1996. Tumour promotion by cyanobacterial toxins. *Phycologia*, 35:74–79.

Ferrão-Filho, A.S., M.F.O. Azevedo, and W.R. DeMott. 2000. Effects of toxic and nontoxic cyanobacteria on the life history of tropical and temperate cladocerans. *Freshwater Biol.*, 45:1–19.

Fogg, G.E., W.D.P. Stewart, P. Fay, and A.E. Walsby. 1973. *The blue-green algae.* Burlington, Mass.: Academic Press.

Foy, R.H., C.E. Gibson, and R.V. Smith. 1976. The influence of daylength, light intensity and temperature on the growth rates of planktonic blue-green algae. *Br. Phycol. Jour.*, 11:151–163.

Ganf, G.G., and R.L. Oliver. 1982. Vertical separation of light and available nutrients as a factor causing replacement of green algae by blue-green algae in the plankton of a stratified lake. *Jour. Ecol.*, 70:829–844.

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Gilbert, J.J. 1994. Susceptibility of planktonic rotifers to a toxic strain of *Anabaena flos-aquae*. *Limnol. Oceanogr.*, 39:1286–1297.

Gliwicz, Z.M., and W. Lampert. 1990. Food thresholds in *Daphnia* species in the presence of blue-green filaments. *Ecology*, 71:691–702.

Graham, L.E., and L.W. Wilcox. 2000. *Algae*. Upper Saddle River, N.J.: Prentice Hall.

Gugger, M.F., and L. Hoffmann. 2004. Polyphyly of true branching cyanobacteria (Stigonematales). *Int. Jour. Syst. Evol. Microbiol.*, 54:349–357.

Gugger, M., C. Lyra, P. Henriksen, A. Couté, J-F. Humbert, and K. Sivonen. 2002. Phylogenetic comparison of the cyanobacterial genera *Anabaena* and *Aphanizomenon*. *Int. Jour. Syst. Evol. Microbiol.*, 52:1867–1880.

Henson, B.J., S.M. Hesselbrock, L.E. Watson, and S.R. Barnum. 2004. Molecular phylogeny of the heterocystous cyanobacteria (subsections IV and V) based on *nifD*. *Int. Jour. Syst. Evol. Microbiol.*, 54:493–497.

Hoffmann, L., J. Komárek, and J. Kastovsky. 2005. System of cyanoprokaryotes (cyanobacteria)—State in 2004. *Algol. Stud.*, 117:95–115.

Horne, A.J., and M.L. Commins. 1987. Macronutrient controls on nitrogen fixation in planktonic cyanobacterial populations. *NZJ Mar. Freshwater Res.*, 21:413–423.

Hrudey, S., M. Burch, M. Drikas, and R. Gregory. 1999. Remedial measures. In *Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management*. I. Chorus and J. Bartram, eds. London: E&FN Spon.

Huisman, J., and F.D. Hulot. 2005. Population dynamics of harmful cyanobacteria: Factors affecting species composition. In *Harmful cyanobacteria*. J. Huisman, H.C.P. Mathis, and P.M. Visser, eds. New York: Springer.

Jardine, C., N. Gibson, and S. Hrudey. 1999. Detection of odour and health risk perception of drinking water. *Water Sci. Technol.*, 40:91–98.

Kirk, J.T.O. 1983. *Light and photosynthesis in aquatic ecosystems*. Cambridge, UK: Cambridge University Press.

Komárek, J. 2003a. Coccoid and colonial cyanobacteria. In *Freshwater algae of North America*. J.D. Wehr and R.G. Sheath, eds. Maryland Heights, Mo.: Elsevier Science.

Komárek, J. 2003b. Filamentous cyanobacteria. In *Freshwater algae of North America*. J.D. Wehr and R.G. Sheath, eds. Maryland Heights, Mo.: Elsevier Science.

Komárek, J. 2005. The modern classification of cyanoprokaryotes (cyanobacteria). *Oceanolog. Hydrobiol. Stud.*, 34:5–17.

Lampert, W. 1981. Toxicity of the blue-green *Microcystis aeruginosa*: Effective defense mechanism against grazing pressure by *Daphnia*. *Verh. Int. Verein. Limnol.*, 21:412–427.

Maier, H.R., M.D. Burch, and M. Bormans. 2001. Flow management strategies to control blooms of the cyanobacterium *Anabaena circinalis*, in the River Murray at Morgan, South Australia. *Regul. Rivers Res. Manage.*, 17:637–650.

Mazur-Marzec, H. 2006. Characterization of phycotoxins produced by cyanobacteria. *Oceanolog. Hydrobiol. Stud.*, 35:85–109.

McGuire, M. 1995. Off-flavor as the consumer's measure of drinking water safety. *Water Sci. Technol.*, 31:1–8.

Newcombe, G. 2002. *Removal of algal toxins from drinking water using ozone and GAC*. Denver, Colo.: Awwa Research Foundation.

Oliver, R.L., and G.G. Ganf. 2000. Freshwater blooms. In *The ecology of cyanobacteria*. B.A. Whitton and M. Potts, eds. Dordrecht, Netherlands: Kluwer Academic Publishers.

Otsuka, S., S. Suda, R. Li, M. Watanabe, H. Oyaizu, S. Matsumoto, and M.M. Watanabe. 1999. Phylogenetic relationships between toxin and non-toxic strains of the genus *Microcystis* based on 16S to 23S internal transcribed spacer sequence. *FEMS Microbiol. Lett.*, 172:15–21.

Otsuka, S., S. Suda, S. Shibata, H. Oyaizu, S. Matsumoto, and M.M. Watanabe. 2001. A proposal for the unification of five species of the cyanobacterial genus *Microcystis* Kützing *ex* Lemmermann 1907 under the rules of the bacteriological code. *Int. Jour. Syst. Evol., Microbiol.* 51:873–879.

Padisak, J. 1998. Sudden and gradual responses of phytoplankton to global climate change: Case studies from two large, shallow lakes (Balaton, Hungary, and the Neusiedlersee, Austria/Hungary). In *Management of lakes and reservoirs during global change*. D.G. George, J.G. Jones, P. Puncochar, C.S. Reynolds, and D.W. Sutcliffe, eds. Dordrecht: Kluwer Academic Publishers. pp. 111–125.

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Paerl, H.W. 1988. Growth and reproductive strategies of freshwater blue-green algae (cyanobacteria). In *Growth and reproductive strategies of freshwater phytoplankton.* C.D. Sandgren, ed. Cambridge, UK: Cambridge University Press.

Piehler, M.F. 2008. Watershed management strategies to prevent and control cyanobacterial harmful algal blooms. In *Advances in experimental medicine and biology,* Vol. 619: *Cyanobacterial harmful algal blooms, state of the science and research needs.* H.K. Hudnell, ed. New York: Springer.

Preussel, K., A. Stuken, C. Wiedner, I. Chorus, and J. Fastner. 2006. First report on cylindrospermopsin producing *Aphanizomenon flos-aquae* (cyanobacteria) isolated from two German lakes. *Toxicon,* 47:156–162.

Rajaniemi, P., P. Hrouzek, K. Kaštovská, R. Willame, A. Rantala, L. Hoffmann, J. Komárek, and K. Sivonen. 2005. Phylogenetic and morphological evaluation of the genera *Anabaena, Aphanizomenon, Trichormus* and *Nostoc* (Nostocales, cyanobacteria). *Int. Jour. Syst. Evol. Microbiol.,* 55:11–26.

Rolland, A., D.F. Bird, and A. Giani. 2005. Seasonal changes in compostion of the cyanobacterial community and the occurrence of hepatotoxic blooms in the eastern townships, Quebec, Canada. *Jour. Plank. Res.,* 27:683–694.

Rother, J.A., and P. Fay. 1979. Blue-green algal growth and sporulation in response to simulated surface bloom conditions. *Br. Phycol. J.,* 14:59–68.

Seifert, M., G. McGregor, G. Eaglesham, W. Wickramasinghe, and G. Shaw. 2007. First evidence for the production of cylindrospermopsin and deoxy-cylindrospermopsin by the freshwater benthic cyanobacterium *Lyngbya wollei* (Farlow *ex* Gomont) Speziale and Dyck. *Harmful Algae,* 6:73–80.

Soranno, P.A. 1997. Factors affecting the timing of surface scums and epilimnetic blooms of blue-green algae in a eutrophic lake. *Can. Jour. Fish. Aquat. Sci.,* 54:1965–1975.

Stal, L.J. 2000. Cyanobacterial mats and stromatolites. In *The ecology of cyanobacteria.* B.A. Whitton and M. Potts, eds. Dordrecht, Netherlands: Kluwer Academic Publishers.

Stanier, R.Y., W.R. Sistrom, T.A. Hansen, B.A. Whitton, R.W. Castenholz, N. Pfennig, V.N. Gorlenko, E.N. Kondratieva, K.E. Eimhjellen, R. Whittenbury, R.L. Gherna, and H.G. Truper. 1978. Proposal to place nomenclature of cyanobacteria (blue-green algae) under rules of international code of nomenclature of bacteria. *Int. Jour. Syst. Bacteriol.,* 28:335–336.

Tilman, D., R. Kiesling, R.W. Sterner, S.S. Kilham, and F.A. Johnson. 1986. Green, blue-green and diatom algae: Taxonomic differences in competitive ability for phosphorus, silicon and nitrogen. *Arch. für Hydrobiol.,* 4:473–485.

van Dok, W., and B.T. Hart. 1997. Akinete germination in *Anabaena circinalis* (cyanophyta). *Jour. Phycol.,* 33:12–17.

Visser, P., B.W. Ibelings, L.R. Mur, and A.E. Walsby. 2005. The ecophysiology of the harmful cyanobacterium *Microcystis*: Features explaining its success and measures for its control. In *Harmful cyanobacteria.* J. Huisman, H.C.P. Mathis, and P.M. Visser, eds. New York: Springer.

Walsby, A.E. 1994. Gas vesicles. *Microbiol. Rev.,* 58:94–144.

Watson, S.B. 2003. Cyanobacterial and eukaryotic algal odour compounds: Signals or by-products? A review of their biological activity. *Phycologia,* 42:332–350.

Whitton, B.A., and M. Potts. 2000. Introduction to the cyanobacteria. In *The ecology of cyanobacteria.* B.A. Whitton and M. Potts, eds. Dordrecht, Netherlands: Kluwer Academic Publishers.

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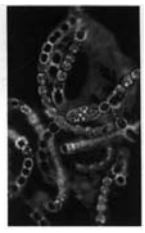

Chapter 6

Chlorophyta

Ann St. Amand

BIOLOGY

Chlorophytes (green algae) are photosynthetic eukaryotes that get their name from the often grass-green color of the cells or colonies, very similar to higher plants. The chlorophytes include many macroalgae such as *Chara* (stonewort or musk grass) that look amazingly close to the land plants (Figure 6-1), and in fact are the closet living relatives of the land plants (Graham 1993, 1996). They are a highly diverse, large group of organisms, with hundreds of genera and thousands of species represented in freshwater, brackish, marine, and terrestrial (including aerial) environments, both attached to substrates and suspended in the water column. Although not generally found in great numbers in thermally impacted habitats such as hot springs, they are common components in snow algal communities, in arctic and Antarctic habitats (freshwater and marine), as symbionts within lichens, as endophytes within coral matrices and seaweeds, and as endosymbionts in freshwater protozoa (Happey-Wood 1988, Graham and Wilcox 2000). This chapter will focus mainly on the freshwater representatives of the chlorophytes.

Chlorophytes are important, generally desirable ecological constituents of the environment, but they can also cause a variety of problems such as excessive blooms that choke off available habitat and impair human recreational use in eutrophic systems (e.g., *Hydrodictyon*), clog water treatment filtration or irrigation equipment (e.g., *Stigeoclonium*; AWWA 2004), as well as cause a variety of taste and odor issues such as the garlic taste generated by *Chara* and the septic taste and odor produced by *Cladophora*. Chlorophytes are pictured in Figure 6-2 (Mallevialle and Suffet 1987). All of these issues can create management and treatment challenges that result in inconsistent water quality and higher operational costs for reservoirs and drinking water or industrial facilities.

All chlorophytes have several characteristics in common, an indication of their common ancestry: they have at least one plastid and are all considered autotrophic to some extent; they have flagella in at least one life stage; they have stellate structures at the flagellar transition zone; the chloroplast is bound by a two-membrane envelope; the chlorophylls are located in the thylakoids; and they all produce and store starch within the chloroplast (often within specialized structures called pyrenoids, Figure 6-3),

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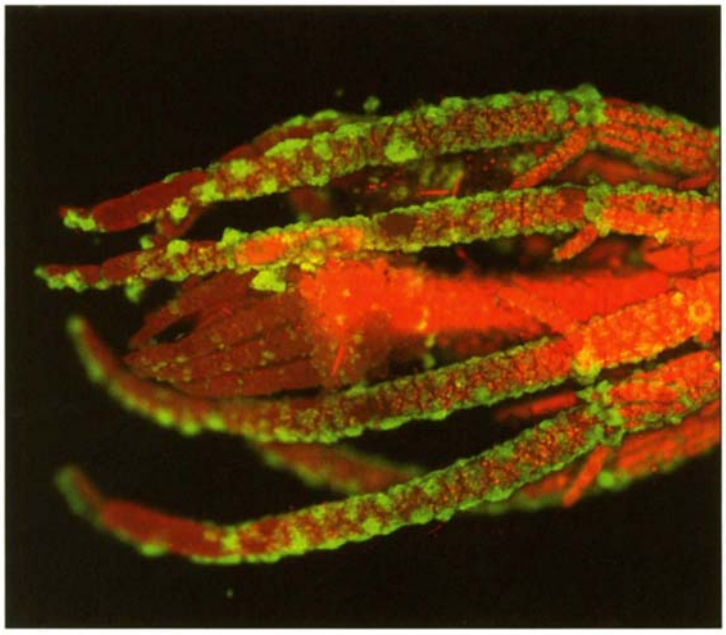

Figure 6-1 *Chara*

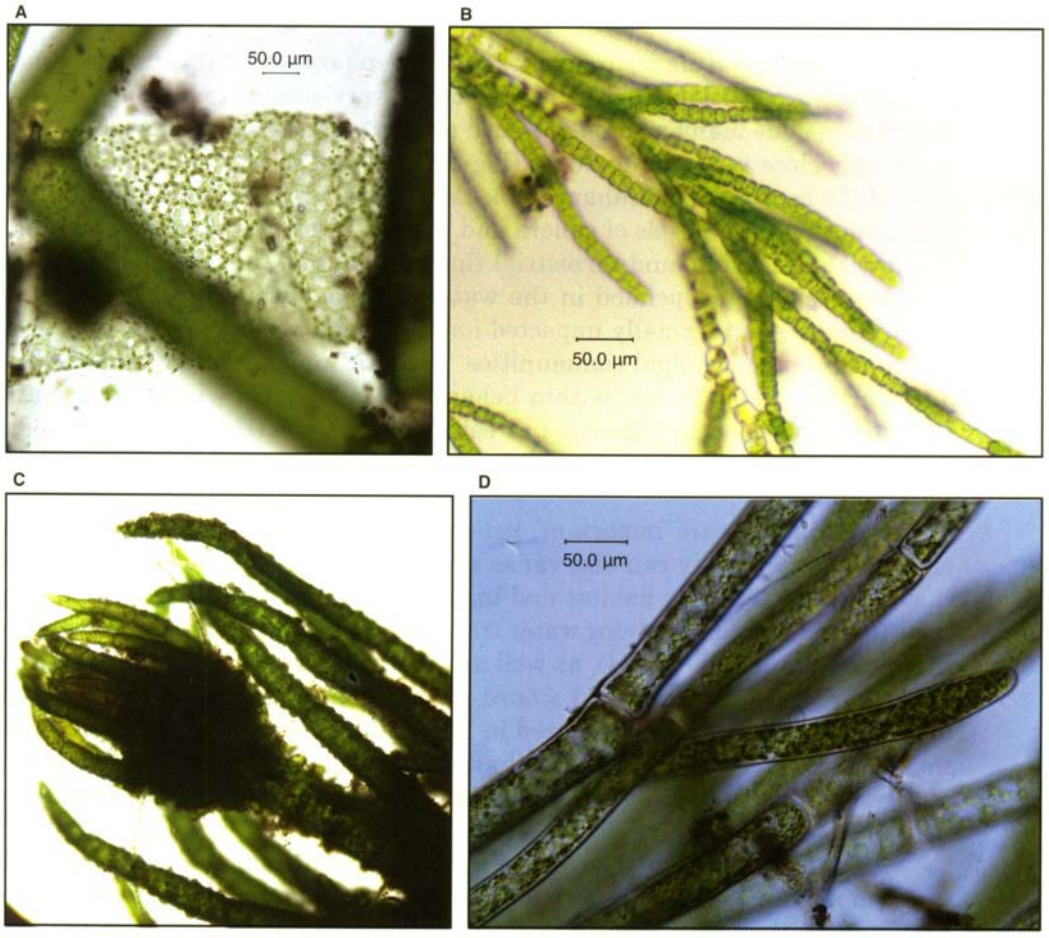

Figure 6-2 (A) *Hydrodictyon*, (B) *Stigeoclonium*, (C) *Chara*, (D) *Cladophora*

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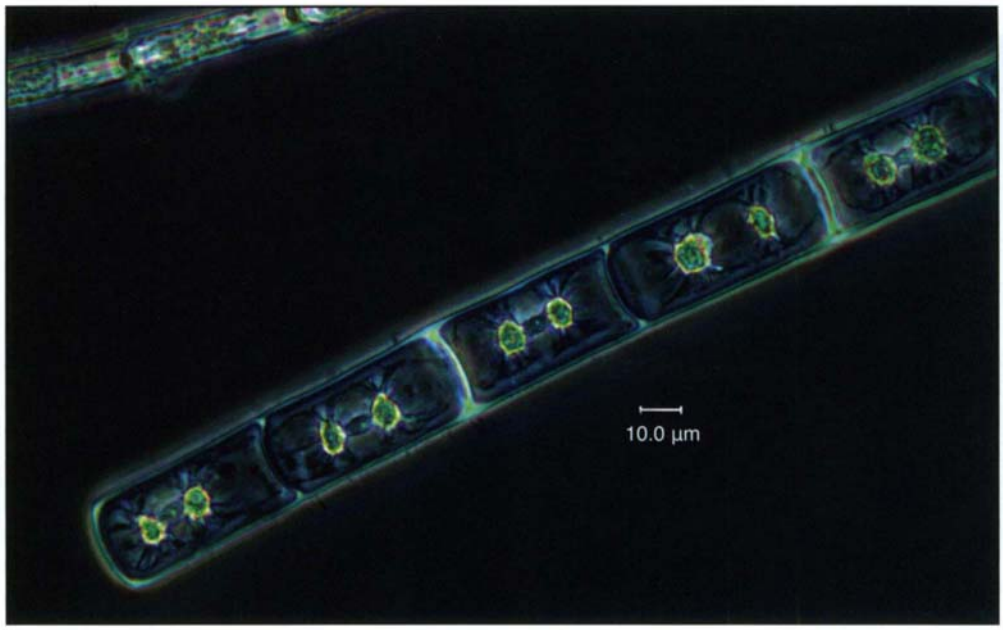

Figure 6-3 Pyrenoid (*Zygnema*)

which is unique to the chlorophytes (Graham and Wilcox 2000). The main pigments in chlorophytes include chlorophylls *a* and *b*, although they do have a host of accessory, primarily carotenoid pigments such as lutein, as well (Wehr and Sheath 2003). Accessory pigments help protect the chlorophylls from the formation of toxic forms of oxygen, provide protective shading, and extend the range of wavelengths that are available for use in photosynthesis (Bold and Wynne 1985, Graham and Wilcox 2000).

Morphologically, chlorophytes range from single cells measuring microns in length to macroscopic, plantlike thalli measuring meters in length. There are single-celled and colonial motile genera containing one, two, four, or many flagella per cell (John et al. 2002). Examples of flagellated single cells include the largest genus within the chlorophytes, *Chlamydomonas,* with two flagella per cell (Figure 6-4a). Also very common is *Tetraselmis* (Figure 6-4b) with four flagella, and a host of smaller, rarer genera with single flagella including *Pedinomonas* and *Monomastix.* Colonial motile genera include the familiar *Volvox* (Figure 6-5), the first algal genus observed by van Leeuwenhoek in 1700 (Dobell 1932). Also common among the colonial motile genera are *Eudorina* and *Pandorina* (Figure 6-5), all with two flagella per cell that extend out through a colonial sheath. The other main morphological groups include the nonmotile coccoid and colonial chlorophytes such as *Oocystis*, *Scenedesmus*, and *Pediastrum* (Figure 6-6). Colonial genera may have no mucilaginous sheath (*Ankistrodesmus*); an even, relatively well-defined sheath (*Sphaerocystis*); or copious, thick lumpy mucilage (*Botryococcus,* which also has oil as one of its storage products), all illustrated in Figure 6-7. Nonmotile genera include the beautifully symmetric desmids represented by *Micrasterias* and *Closterium* (Figure 6-8). Lastly, the filamentous chlorophytes include both branched (*Cladophora*, *Chara*, *Bulbochaete*) and unbranched (*Sprirogyra, Microspora*, *Ulothrix;* Figure 6-9) genera. Many of the filamentous taxa have specialized holdfast cells that serve to anchor the filament to the substrate.

Reproduction within the chlorophytes varies considerably and involves either asexual or sexual reproduction (Bold and Wynne 1985). In some groups, there is both sexual and asexual reproduction based at least partially on growth cycle, environmental conditions, and season. Asexual reproduction occurs when cells divide and grow

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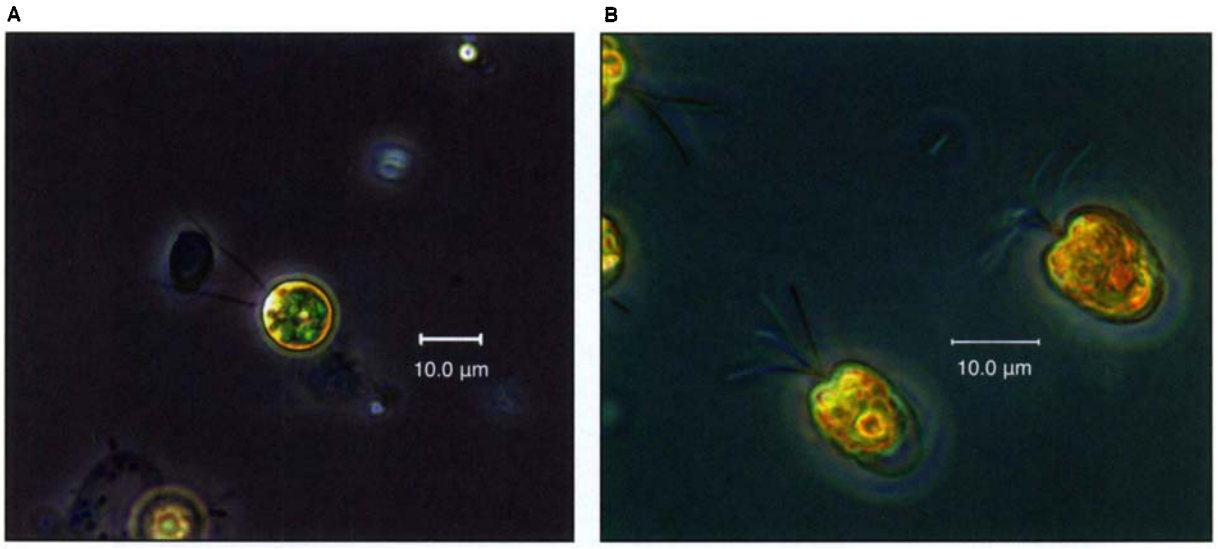

Figure 6-4 (A) *Chlamydomonas*, (B) *Tetraselmis* (*Pyramichlamys*)

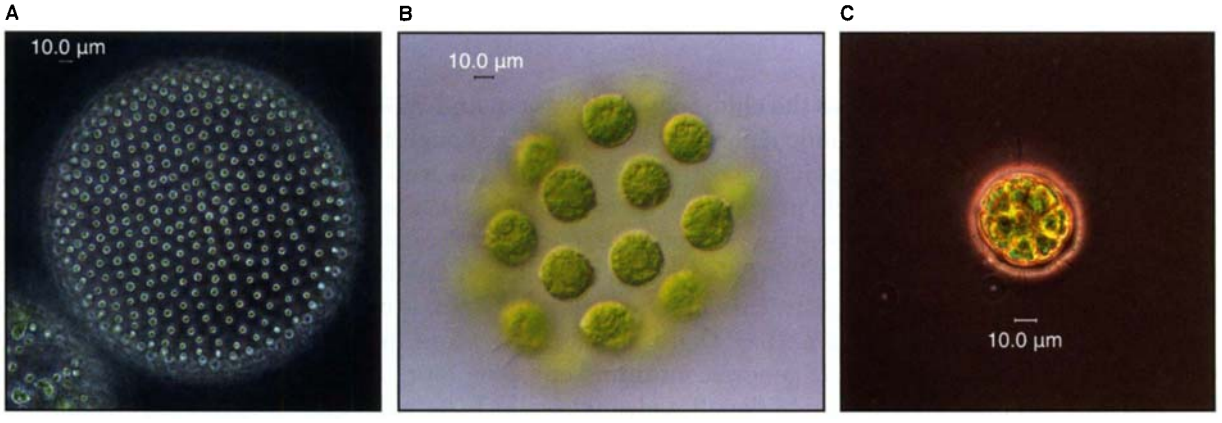

Figure 6-5 (A) *Volvox*, (B) *Eudorina*, (C) *Pandorina*

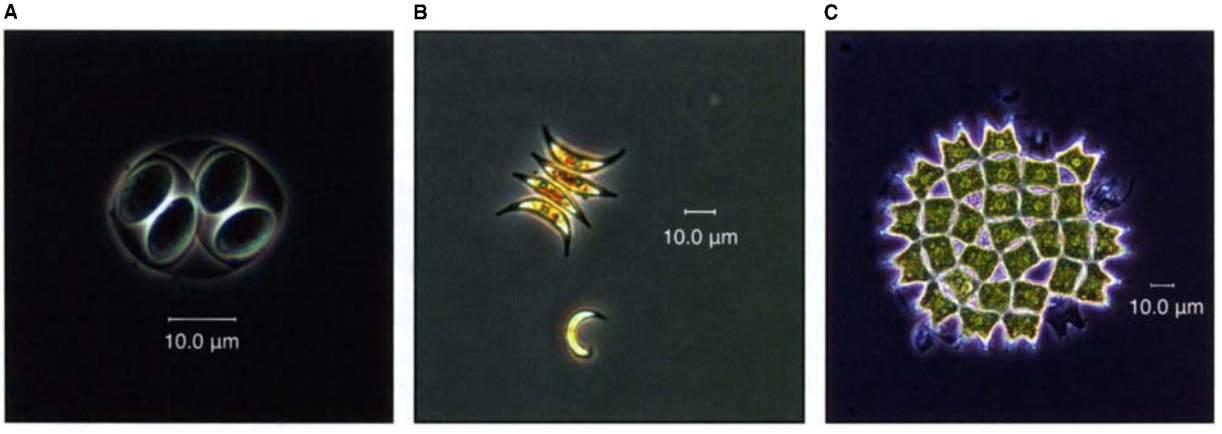

Figure 6-6 (A) *Oocystis*, (B) *Scenedesmus*, (C) *Pediastrum*

without recombining genetic material. At its simplest, cells divide asexually by binary fission and then grow to full size again. Asexual reproduction includes binary fission, forming a variety of aplanospores and autospores, and asexual motile zoospore formation. Autocolony formation occurs in genera like *Scenedesmus* and *Pediastrum*, where

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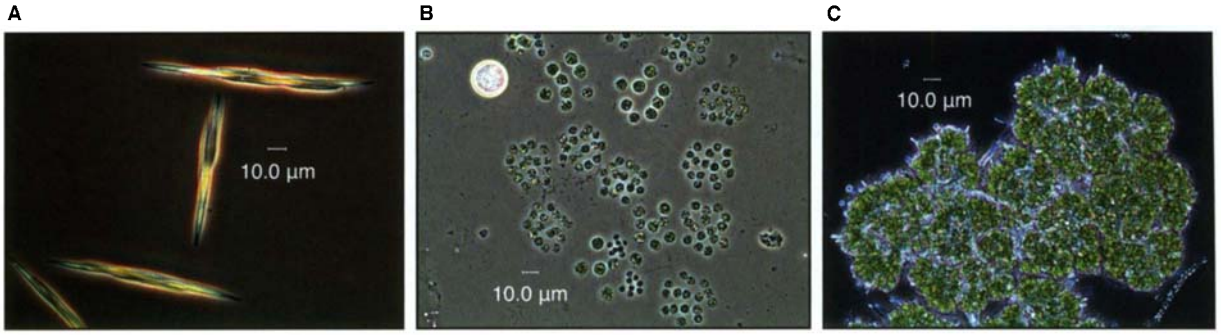

Figure 6-7 (A) *Ankistrodesmus*, (B) *Sphaerocystis*, (C) *Botryococcus*

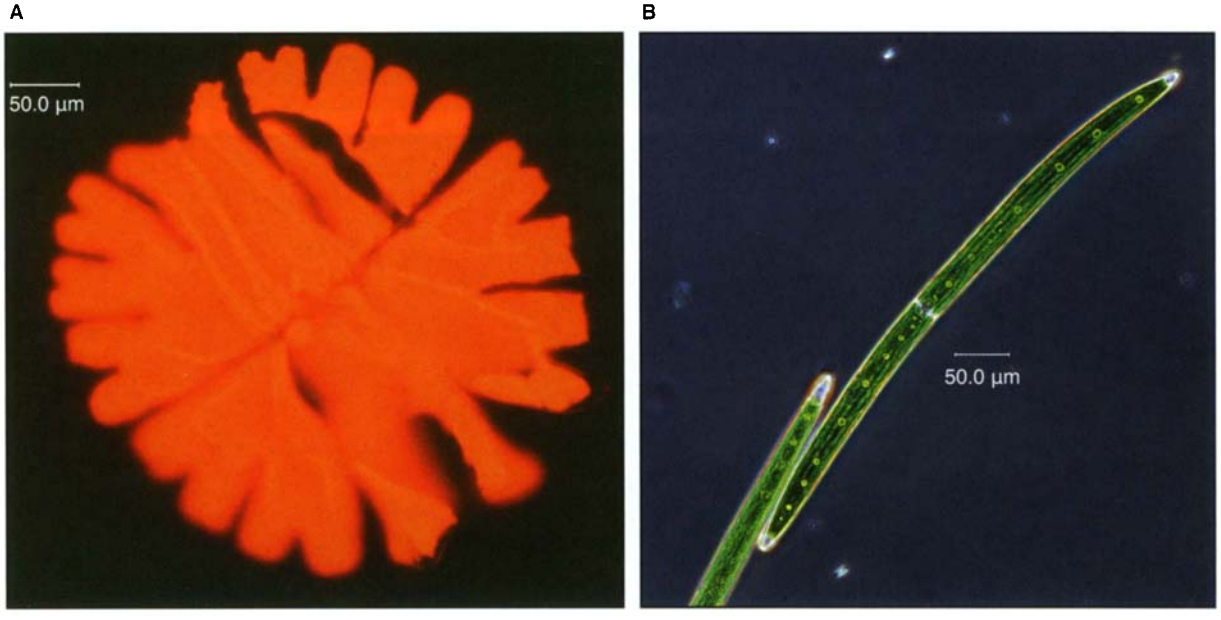

Figure 6-8 (A) *Micrasterias*, (B) *Closterium*

individual cells divide by binary fission and develop into miniature colonies (Graham and Wilcox 2000).

Sexual reproduction involves recombination of genetic material and formation of gametes. Many chlorophytes exhibit a haplontic life cycle (van den Hoek et al. 1995). An example is *Chlamydomonas*, which forms positive and negative gametes (sometimes where the organisms are indistinguishable from the gametes) and only the nucleus of the zygote is diploid. In several genera, the zygote is also a hypnozygote, which is a thick-walled resting cell that germinates after a dormant period. Some genera exhibiting sexual reproduction have vegetative cells that function as gametangia and form oogonium (e.g., *Oedogonium*, which also undergoes noticeable asexual reproduction showing growth rings at the ends of vegetative cells, Figure 6-10). Zygote formation can be observed often with the empty gametangial cell walls still attached as in *Cosmarium* (Figure 6-11). A large group of chlorophytes, the filamentous conjugating or Zygnematales algae, reproduce by fusion of amoeboid gametes (e.g., *Mougeotia*, Figure 6-12) when two filaments unite. Most of the genera that can reproduce sexually also reproduce asexually at some point during their life cycles.

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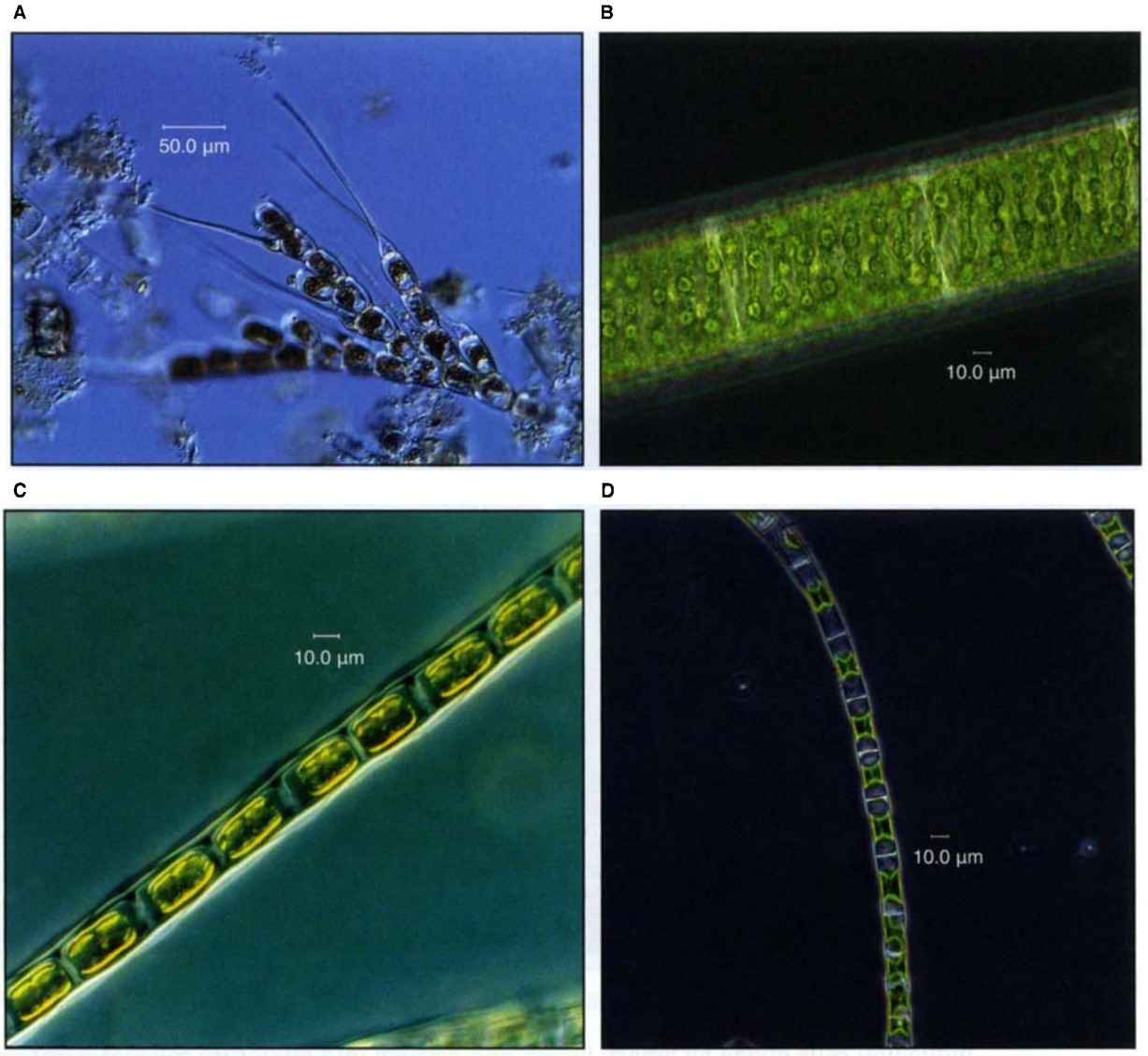

Figure 6-9 (A) *Bulbochaete*, (B) *Spriogyra*, (C) *Microspora*, (D) *Ulothrix*

TAXONOMY

Traditionally, as with most of the other algal groups, chlorophytes have been classified morphologically (e.g., Smith 1950, Prescott 1982). It is considered that they came from a common ancestor but have diverged into many different phylogenic groups with the wide diversity of morphological types noted above (Graham and Wilcox 2000). Until recently, chlorophytes were grouped into approximately 16 orders with the unicellular flagellates, volvocalean algae, unbranched filaments, branched filaments, desmids, and other algae classified into distinct orders (Bold and Wynne 1985).

As in the other algal divisions such as the cyanophyta, there have been substantial revisions in chlorophyte taxonomy over the last 20 years. Chlorophyte taxonomy is now based not only on morphology but also on ultrastructural analyses of the flagellar apparatus, mitosis, cytokinesis, comparative biochemistry, life-history studies, and molecular sequencing (Graham and Wilcox 2000, van den Hoek et al. 1995). Results have indicated multiple examples of convergent evolution where similar-looking

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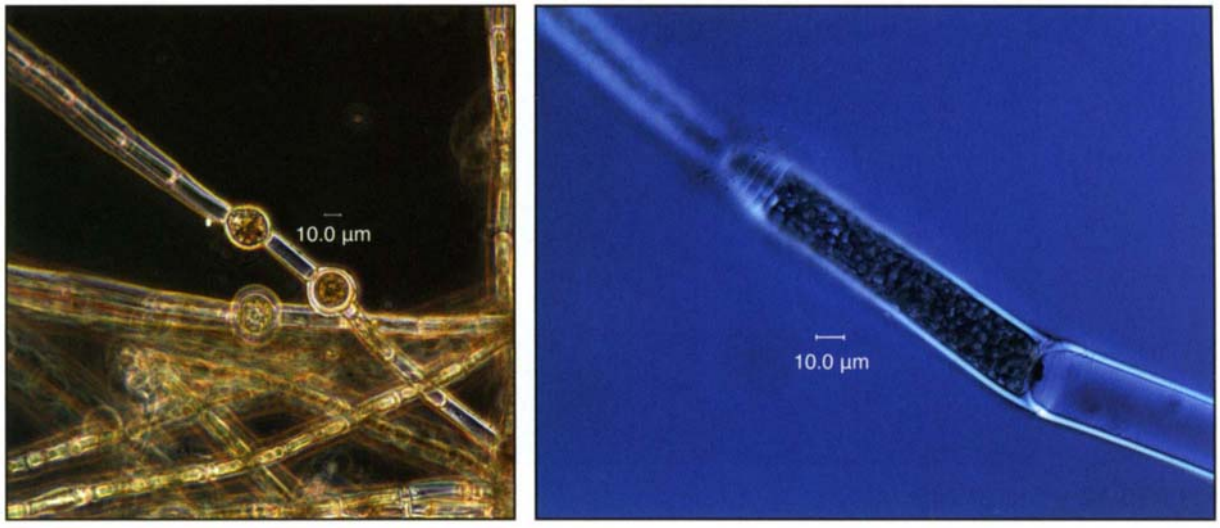

Figure 6-10 *Oedogonium*

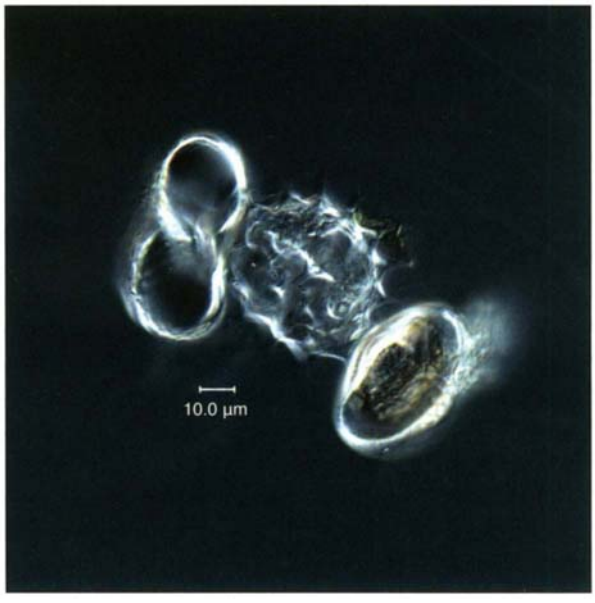

Figure 6-11 *Cosmarium*

species are not closely related phylogenically (Graham and Wilcox 2000). The newer taxonomy divides the chlorophytes into two major lineages and several clades (Mishler et al. 1994) with the major classes being the Chlorophyceans, the Trebouxiophyceans, the Ulvophyceans, the Prasinophyceans, and the Charaphyceans (Mishler et al. 1994, Graham and Wilcox 2000, Wehr and Sheath 2003).

There is still a fair amount of work being done on the taxonomy of the chlorophytes. The Volvocales still contains nonrelated taxa (Buchheim et al. 1994). John et al. (2002) place the green algae into phyla rather than divisions and distinctly separate the Prasinophyta (some of the flagellated chlorophytes) and the Chlorophyta (the rest of the flagellated chlorophytes and branched/unbranched filamentous greens, etc.), as well as reseparate *Chara* and *Nitella* from the Zygnematales. The *Integrated Taxonomic Information System* (www.itis.gov, United States Geological Survey), an online taxonomic listing of all plants, animals, fungi, and bacteria in North America, still

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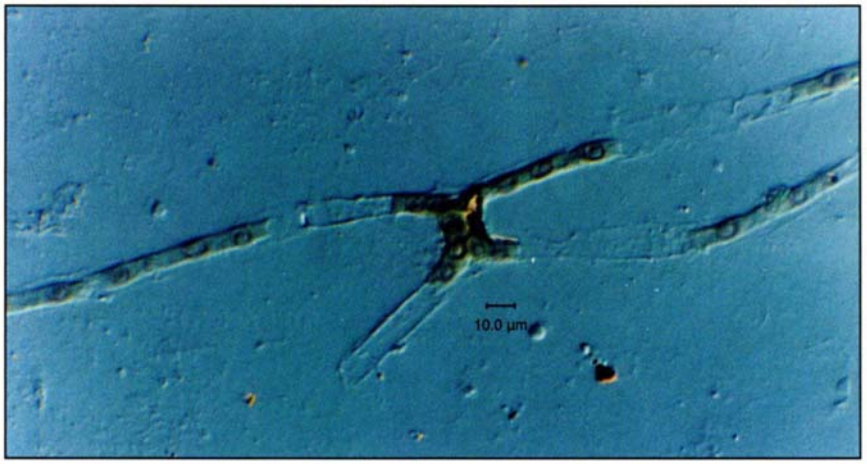

Figure 6-12 *Mougeotia*

refers most consistently to the algal classification scheme described in Bold and Wynne (1985). Although there are as many ways to classify the chlorophytes as there are texts and publications discussing it, especially as new research becomes available, this chapter will focus on the scheme described in Graham and Wilcox (2000) and Wehr and Sheath (2003).

ECOLOGY

Chlorophytes are present to some extent in most surface waters, either in the plankton (suspended algae) or periphyton (attached algae) or both. It can be hard to characterize their basic ecology because the division as a whole is highly diverse. They are common in drinking water supplies, and dominance by chlorophytes usually indicates good water quality, if the taxa present do not produce taste and odor compounds or clog filters or water ways. There are several size classifications that are commonly used when referring to all the plankton: picoplankton (0.2–2 µm), nanoplankton (2–20 µm), netplankton (20–100+ µm) as discussed in Happey-Wood (1988) and Sournia (1980). Macroalgae (100+ µm, generally macroscopic and visible to the naked eye) refers to taxa that can be easily observed as floating or attached colonies with easily distinguishable filaments. These size classes help us to more accurately assess the impacts of different growth and loss processes that are often size and morphology dependent.

Chlorophytes are usually most abundant in early spring through late summer, and less so in late fall and winter when the diatoms and chrysophytes are more abundant. Smaller-celled chlorophytes are likely to be present but do not dominate the plankton of oligotrophic lakes year-round, such as Lake Ontario (Munawar and Munawar 1976). Often, the Volvocales are the first chlorophytes to bloom in spring generally following the spring diatom bloom, where one can often find abundant *Volvox*, *Eudorina*, *Pandorina*, *Pyramichlamys,* and *Chlamydomonas*. As with the other algal groups, population fluctuations throughout the growing season are always moderated by competition for resources, primarily nutrients (Sommer 1989) and light (Kirk 1983), among other resources. *Spirogyra* and *Oedogonium* and several of the other nonbranching filamentous chlorophytes often bloom in late spring, into early summer. Several of the unicellular and small colonial taxa become abundant in midsummer, including *Ankistrodesmus*, *Scenedesmus*, *Oocystis*, *Golenkinia,* and *Sphaerocystis* along with many of the desmid genera in more acidic systems. Lastly, the filamentous branching chlorophytes such as *Cladophora* become more abundant in mid-late summer.

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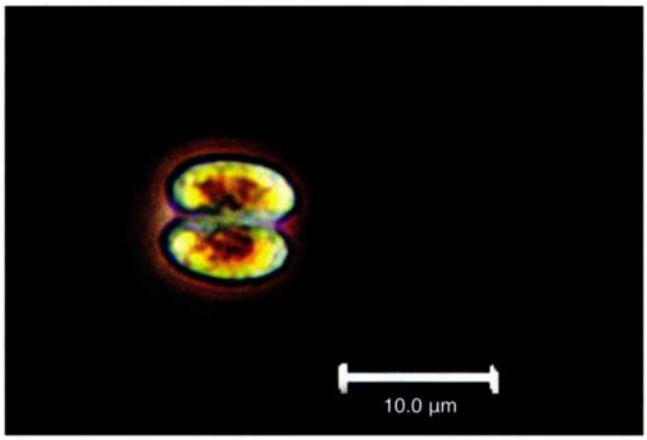

Figure 6-13 *Scenedesmus*

Seasonal oscillations are impacted by a number of different environmental factors including light availability, temperature, flow regime (streams, rivers, and reservoirs), nutrient regime, and for periphytic assemblages, substrate type (Lowe and LaLiberte 2006). Biological factors that impact chlorophyte ecology include competition for light and nutrients, grazing and physiological growth, and mortality. Any particular population or community response will be based on the balance between growth and loss processes as a response to different environmental and biological factors, which will be discussed below (Reynolds 1984, Happey-Wood 1988).

Growth processes include physiological growth from productivity, entrainment from the sediments, recruitment from upstream populations, airborne transport, flooding, or migratory waterfowl. In some cases, grazing, generally a loss process, can result in growth as either a release from competition or direct benefit from passing through grazer guts with resistant sheaths or cell walls and being enriched (Stutzman 1995, St. Amand 1990, Lehman and Sandgren 1985). Physiological growth or productivity tends to be greatest at intermediate light and temperatures for the chlorophytes as chlorophylls can be bleached at very high light intensities (Reynolds 1984), even for primarily benthic taxa like *Cladophora*. Although not directly physiological growth, populations can be augmented by colonies or cells being resuspended from the sediments during spring turnover or storm events, or from sloughing of attached colonies in established benthic mats (Happey-Wood 1988). Chlorophytes tend to prefer higher nitrogen to phosphorus ratios and do well in the presence of high nitrates (Turpin 1988) but also do well in the presence of high nutrients in general. Very high nitrogen and low light conditions resulting from high particulates that are common in many reservoir systems, especially in the southwest, tend to encourage highly abundant but smaller-celled chlorophytes in the Chlorophyceae (e.g., *Scenedesmus*, *Pediastrum*, *Oocystis*, *Crucigenia*) (St. Amand, unpublished data; Figure 6-13).

Loss processes include physiological death or mortality, scouring, hydraulic washout, sinking, and grazing. Physiological death is most common when cells or colonies do not have optimal growth conditions, such as at the end of the growth season or when nutrients become limiting. Fungal infection can also hasten physiological death (Van Donk 1989). Scouring is a physical process that happens when periphytic or attached communities are physically disrupted by animals or flood events. Hydraulic washout is similar to scour but related to high flow in reservoir systems or high-turnover lakes where algae are physically removed to downstream systems. Chlorophytes that require turbulence to stay suspended in the water column will sink out when the lake sets up in the summer or the turbulence subsides, and faster sinking rates are often

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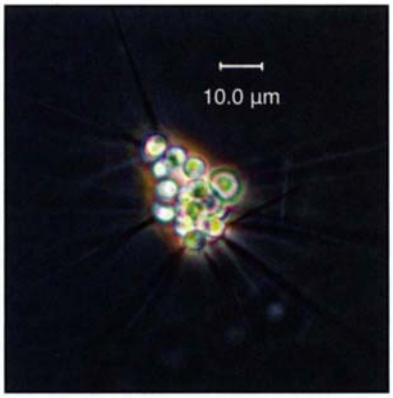

Figure 6-14 *Micractinium*

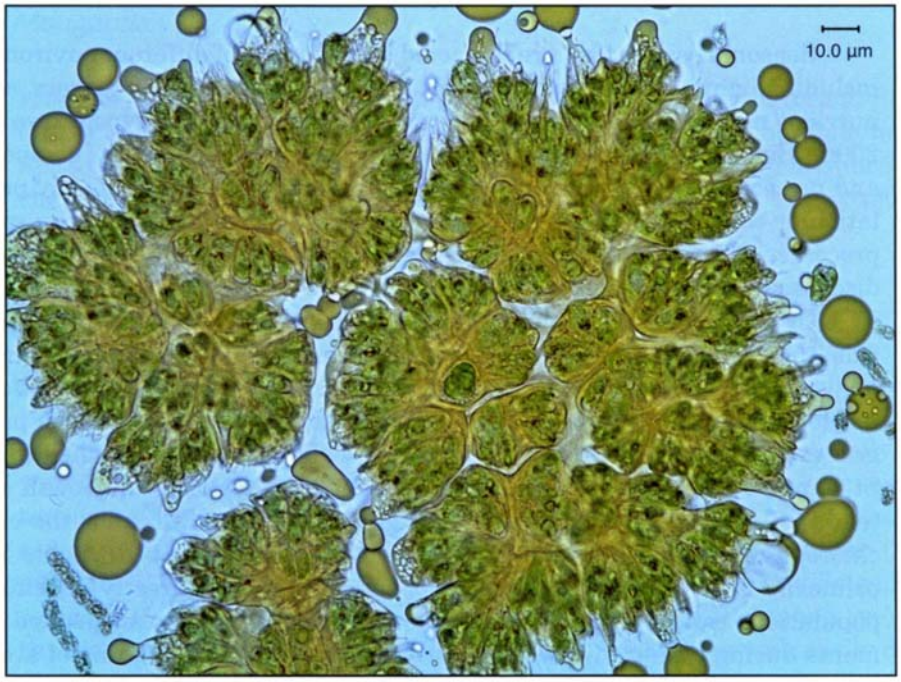

Figure 6-15 *Botryococcus*

related to poor physiological health (Reynolds 1984). Large setae or gelatinous extensions can often help cells maintain position up in the water column such as in *Micractinium* (Figure 6-14) as can alternative storage products such as the oil *Botryococcus* produces. The oil in *Botryococcus* also has potential as an alternative biodiesel source (Figure 6-15; Graham and Wilcox 2000). *Spirogyra* and *Oedogonium* mats can trap oxygen bubbles, keeping the diffuse mats floating and suspended in the water as well. This is a double-edged sword because the filaments closest to the surface may bleach and die due to high light (Holdren et al. 2001).

The last lost process, and probably the most complicated biologically, is loss due to grazing. There are essentially four common responses to grazing, which reflect both direct and indirect effects (Lehman and Sandgren 1985, Sterner 1989). Taxa tend to either decrease linearly, have no response to grazing, increase at low grazer densities and decrease at high grazer densities, or linearly increase (species like *Sphaerocystis* mentioned above that pass through the gut enriched rather than digested; Figure 6-16).

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Figure 6-16 *Sphaerocystis*

Grazer size and type affects how efficient grazers are in any particular assemblage. In general, any phytoplankton algal unit below 20 µm is edible, but this size threshold is highly grazer and algal morphology dependent (Carpenter et al. 1993). Daphnids are considered general grazers, filtering anything that will fit in its feeding groove, while copepods, especially mostly herbivious calanoid copepods, are more selective in their food choices. Large daphnids (1 mm or above) are the most efficient phytoplankton grazers, while cladocerans such as *Bosmina* are less efficient (Carpenter et al. 1993). Rotifers like *Brachionus* filter feed through ciliated corona and are limited to a much smaller algal taxa like *Pyramichlamys* (*Tetraselmis* Figure 6-17). For periphytic algae, especially the microalgae, the primary grazers are macroinvertebrates such as chironomids, caddis flies, mayflies, snails, and crayfish (Lamberti et al. 2006). Several of the larger macroalgae, though, are grazer resistant (e.g., *Cladophora*, *Pithophora,* and *Hydrodictyon*), making grazer control unlikely (Sandgren et al. 2004). Grazing on *Cladophora*, for example, is likely limited to the extensive epiphyton growth it supports (Dodds and Gudder 1992).

SIGNIFICANCE

These growth and loss processes are often well balanced, keeping the microalgal chlorophytes from producing nuisance blooms very often (Lembi 2003). Although microalgae can bloom extensively, coloring the water pea green, they most often cause problems when they produce taste and odor compounds (Mallevialle and Suffet 1987). Another issue with small chlorophyte blooms is that a very large standing crop of small cells adds a lot of organics (including taste and odor compounds) to the water, which increases the complexity of treatment, chlorine by-products, and treatment cost.

Several of the filamentous chlorophytes, however, can form conspicuous nuisance mats near or on lake, river, and structure surfaces, severely impairing use (Holdren et al. 2001). *Microspora*, which can overgrow the walls of settling basins quickly, causes problems occasionally. The nonbranching *Spirogyra*, *Rhizoclonium,* and *Mougeotia*

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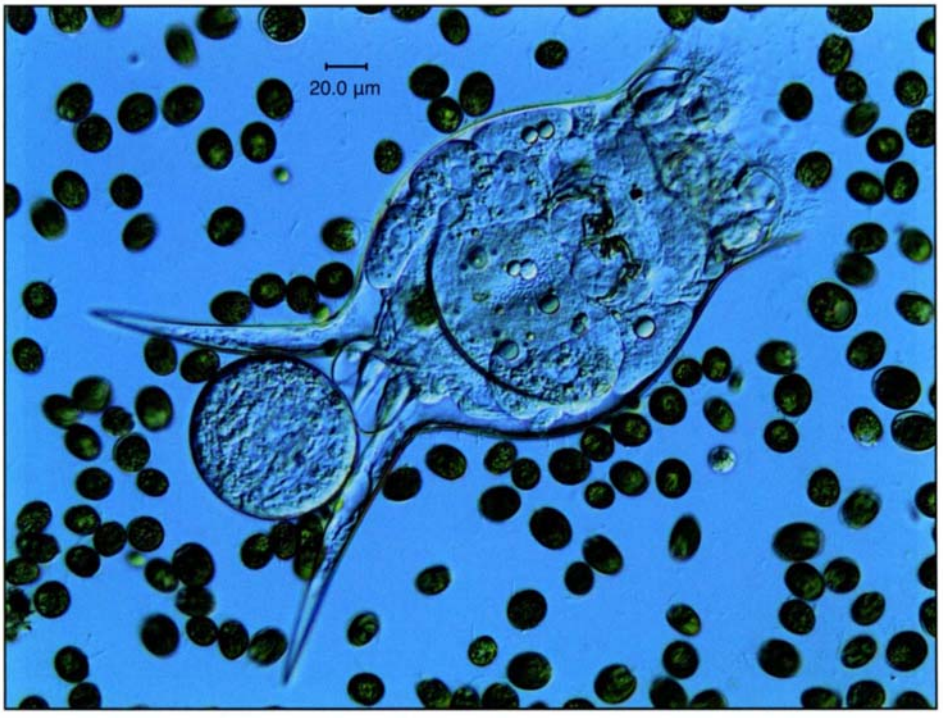

Figure 6-17 *Tetraselmis (Pyramichlamys)*

produce free-floating surface blooms but are somewhat easier to control and are less resistant to chemical control (Lembi 2000). The largest concerns in terms of macroscopic filamentous blooms that cause issues with access to water and water quality include *Hydrodictyon*, *Cladophora*, *Pithophora,* and the stoneworts (usually *Chara*, and to a lesser extent *Nitella* and the invasive *Nitelliopsis*). *Hydrodictyon,* or water net, can accumulate in huge blooms covering large areas, limiting access to swimming and fishing areas, causing low dissolved oxygen when the mats die off, and clogging filters in drinking water plants. Mechanical removal methods are not effective with *Hydrodictyon* (Holdren et al. 2001), and as with many of the robust filamentous chlorophytes, it is tolerant to copper (Lembi 2000). Although *Hydrodictyon* is often associated with high nitrogen concentrations, it grows well at lower nitrogen concentrations, making it a good competitor against the other chlorophyte macroalgal taxa such as *Cladophora* (Wells et al. 1999, Hall and Cox 1995).

Cladophora is another macroscopic chlorophyte that causes major bloom issues. It is primarily a problem as an attached algal nuisance, accumulating enormous amounts of biomass (Stauffer 2004) and impacting filtration equipment and dam operations; it can produce taste and odor compounds as well (AWWA 2004, Mallevialle and Suffet 1987). In systems supporting trout reproduction, it can overgrow and impact fish reproduction and habitat quality (Neil 1975), and it has become an increasingly destructive bloomer in the Great Lakes (Harris 2004, Stauffer 2004). Like *Hydrodictyon* and *Pithophora*, *Cladophora* is largely inedible in its mature state (Sandgren et al. 2004) and does well in elevated nutrient conditions (Higgins 2004). The invasion of *Dreissena* spp. (zebra and quagga mussels) into the Great Lakes has confounded efforts to lower nutrients in the Great Lakes, ultimately increasing nuisance *Cladophora* blooms. *Dreissena* clears the water of potential competitors (*Cladophora* does better at high light intensities) and seems to increase available phosphorus for *Cladophora* growth (Higgens 2004).

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Figure 6-18 *Pithophora*

Pithophora, or horsehair algae (Figure 6-18), is a nuisance mat former that is extremely hard to control and is also associated with elevated nutrient levels. It can interfere with fishing, irrigation, or other water uses. *Pithophora* is extremely chemical resistant (Lembi 2000, 2003) and carries the additional strategy of producing resistant akinetes that can survive harsh environmental conditions (John et al. 2002). Under some circumstances, as with some of the other filamentous chlorophytes, high nitrogen concentrations have been associated with *Pithophora* blooms (Spencer and Lembi 1981). Interestingly, *Pithophora* may have a positive use as a bioabsorbant for heavy metals in wastewater (Priya et al. 2007) and as a removal mechanism for dyes such as malachite green and methylene blue in wastewater as well (Vasanth et al. 2005).

The stoneworts can also substantially impair access, lower diversity, and cause taste and odor issues because of their strong garlic smell, but they do not float up in the water column or transport within the system like *Hydrodictyon* and *Pithophora* do. *Chara* and *Nitella* are often highly desirable components of littoral vegetation communities because of the habitat they provide and the stabilizing effect they have on the sediments (Lembi 2003). *Chara* is also a food source for coots and some species of ducks as well. Because of their larger, more substantial thallus size, they are more easily physically removed than taxa like *Hydrodictyon.* Blooming in more shallow areas where there is good access to light, *Chara* and *Nitella* do not grow well in high phosphorus conditions it seems (Simons et al. 1994), but there are likely other controls on Charophyceae (van den Berg et al. 1998).

IDENTIFICATION

Although identification of the chlorophytes depends on more than morphological characteristics, most taxonomists using the light microscope are analyzing preserved water samples and are restricted to using morphology exclusively (AWWA 2002). There are several general characteristics to look for initially that determine the order, family, or

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group so that a genus and perhaps species identification can be made. It is important to remember that adding preservative will always change some cell traits like true color, cell shape, and perhaps number of flagella (cells may drop flagella on preservation), so having live material available will help tremendously. Also, although all chlorophytes can produce starch as a storage product, not all cells will be producing starch at any one time, especially if they are growing quickly. Lastly, environmental conditions and growth stage can also greatly affect how cells and colonies look when collected. Young cells may be smaller and lack some structures, and young colonies may not look the same as older, more mature colonies (e.g., sheath color, number and location of cells, size).

When assessing morphology, note coloniality first. Generally, there are unicells, colonies (with or without sheath), filaments (unbranched or branched), and plantlike algae (look like vascular plants, or similarly to stoneworts). Taxonomists try to observe as many natural units as possible when trying to confirm an identification, at least 50–100 if they are dominant. The more cells or colonies one can observe, the more likely one is to see the full variation in the taxa. Remember that multiple life stages may be present, so a *Chlamydomonas* cell may be dividing with two or four daughter cells still within the old mother cell wall, making it look colonial. Consider the cell shape (e.g., spherical, pyriform, or ovate) and how the cells are arranged in the colony (e.g., in groups of 2/4/8, random, or peripheral). Some cells (often not all), may have setae or spines that determine their genus, but at times these structures may be fine or short and difficult to see easily at low magnification (less than 400×).

Look for the presence or absence, and number, of flagella. If present, where are the flagella attached? If you suspect that the population may have been motile, but the cells lack flagella, try to look for free flagella in the surrounding water, and if you have access to live material, look for motility in the live cells. Often cells like *Chlamydomonas* may have dropped their flagella, but the insertion point or a papilla is still present. It is also not uncommon for cells to drop some, but not all, of their flagella. Again, looking at as many specimens as you can of each type of algae will help you be confident in your identification.

In filamentous taxa, look for holdfast cells, whether the filaments are branched or not, and the manner of branching (e.g., opposite or alternating, numerous or sparse). These features should help you get it to order or family, and from there you can further identify it to genus. If you have any questions about whether a colony or cell is a chlorophyte or cyanophyte, look for a well-delineated cell wall and organized cell contents. The starch test may or may not be positive for the chlorophytes. Cell attachments are sometimes genus-specific (e.g., *Oedogonium*), so check out the cells both in the middle of, and on the ends of, as many filaments as you can.

Other features to look for include resting cells, zygotes, or oogonium and whether filaments look as if they are getting ready to conjugate (some cells will have a bulge on the side). Chloroplast structure can be quite helpful as well, since many of the important filamentous genera have very different chloroplast structures (e.g., spiral chloroplast in *Spirogyra* vs. a twisted plate in *Mougeotia*). Often, certain genera appear together, so if you have *Oocystis* in abundance, it is likely you might also have *Scenedesmus* and *Ankistrodesmus* to some extent. Also, it is not uncommon for *Pyramichlamys (Tetraselmis)* to co-occur with *Chlamydomonas* and *Pandorina* as well as small centric diatoms.

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KEY TO MORPHOLOGICAL GROUP

Single cell with 2–4 flagella, Go to 1

Multiple cells in colony, with 2 flagella, Go to 3

Multiple cells without flagella, Go to 5

Single cell (or very few cells) without flagella, Go to 7

Multiple cells arranged in branching/nonbranching filaments, filaments not upright or plantlike, Go to 19

Multiple cells arranged in branching filaments, clearly upright and plantlike, filamentous branches large, visible easily to the naked eye, often with a gritty feel, Go to 27

Motile: Single Cells

1a. Single cell spherical or ovoid, with 2 flagella, *Chlamydomonas*

1b. Single cell with more than 2 flagella, Go to 2

2a. Single cell with 4 flagella, slightly dorso-ventrally flattened, flagella inserted into an anterior depression that often gives the cell a heart-shaped or pyriform look, *Tetraselmis* (*Pyramichlamys*)

2b. Multiple cells with 2 flagella, Go to 3

Motile: Multiple Cells per Colony

3a. Cells within a colony in contact with one another, cells pyriform, ≤16 cells/colony, *Pandorina*

3b. Cells within a colony not in contact with one another, Go to 4

4a. Colony composed of 500–50,000 cells. Cells small (approximately spherical), peripheral in the colony, *Volvox*

4b. Colony with ≤32 cells. Cells broadly ovoid or spherical, *Eudorina*

Nonmotile: Single (or Few)/Multiple Cells per Colony

5a. Cells elongate and jointed together to form a net (with openings formed by 3–6 cells), *Hydrodictyon*

5b. Algae not forming a net, Go to 6

6a. Cells randomly spread throughout the colony in groups of 4–8 (up to 64 cells), cells spherical, enclosed within a spherical to ellipsoidal mucilaginous sheath, *Sphaerocysits*

6b. Cells elongated, attached side by side with their long axis parallel to one another. Number of cells commonly 2, 4, or 8, *Scenedesmus*

6c. Cells not attached side by side or within an obvious colony or single, Go to 7

7a. Cells divided into mirror-image halves (semicells) by a mid-region constriction. If constriction not obvious in the cell wall itself, the chloroplast is so divided (Desmids), Go to 8

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7b. Cells not divided at the mid-region, Go to 12

8a. Cells flattened with a deep median constriction semicells with several lobes, those lobes with secondary lobes and lobules that appear as sharp teeth, *Micrasterias*

8b. Cells not flattened, Go to 9

9a. Cell margin with rounded lobes, apices with a deep notch or shallow depression; semicells with one to several protuberances that are often granulate, *Euastrum*

9b. Cells not as above, Go to 10

10a. Each half of cell with three or more spinelike or pointed arms, cells pyramidal, *Staurastrum*

10b. Cells with no such extensions, not pyramidal or several-angled in top view, Go to 11

11a. Cells circular or subcircular in top view, *Cosmarium*

11b. No distinct mid-region constriction in cell wall. Two chloroplasts per cell with unpigmented area across center of cell. Conspicuous vacuole in each apical region. Most species are bowed or crescent shaped. A few have straight cells, *Closterium*

12a. Cells forming filaments, Go to 19

12b. Cells isolated or in nonfilamentous colonies, Go to 13

13a. Cells elongated, Go to 14

13b. Cells round to oval or angular, Go to 16

14a. Cells colonial, radiating from a central point, *Actinastrum*

14b. Cells isolated or in irregular clusters, Go to 15

15a. Cells 5 to 10× as long as broad, crescent shape to almost straight, *Ankistrodesmus*

15b. Cells 2 to 4× as long as broad, semicircular cell apices pointed, *Selenastrum*

16a. Cells solitary (can rarely be found dividing within the old mother cell wall). Cells 3-, 4- or 5-sided, with blunt or round corners, and sometimes terminating in a spine, *Tetraedron*

16b. Cells in a colony of (2) 4–128 angular cells forming a circular plate, marginal cells usually shaped differently than those within the colony and often with 2 projections, *Pediastrum*

16c. Cells in colonies and not arranged as above, Go to 17

17a. Colony at tight, hollow sphere of cells, either connected directly or with short connecting processes between cells, *Coelastrum*

17b. Colony 4-celled or in a series of 4-celled groups, generally not exceeding

16 cells, quadrate and flattened. Cells ellipsoidal, triangular, or rectangular, *Crucigenia*

17c. Colony a loose cluster of cells, sometimes enclosed by a common membrane, Go to 18

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18a. Cells ellipsoid, oval, often with nodules on the ends, 1 to 4 cells (multiple cells encased in the old mother cell wall), *Oocystis*

18b. Cells round to ovate, connected to center of colony by a branching stalk that consists of fragments of the old mother cell walls, *Dictyosphaerium*

18c. Cells round to elllipsoidal, chloroplast cup shaped, and cells isolated or in tightly grouped colony, *Chlorella*

Nonmotile: Cells in Filaments

19a. Filaments branched, Go to 24

19b. Filaments not branched, Go to 20

20a. Chloroplast(s) twisted into spiral (helical) form, *Spirogyra*

20b. Chloroplast not spiral, Go to 21

21a. Chloroplast a flat or twisted axial ribbon with several conspicuous pyrenoids, *Mougeotia*

21b. Chloroplast not as above, Go to 22

22a. Cells cylindrical, with 2 star-shaped chloroplasts each containing a single pyrenoid, *Zygnema*

22b. Chloroplast not as above, Go to 23

23a. Chloroplast a parietal network with several pyrenoids. Cells cylindrical but slightly narrowed at the posterior ends. Some cells with one or more ringlike scars. Filaments often not straight. May have distinctly enlarged oogonia, *Oedogonium*

23b. Chloroplast parietal and reticulate with several pyrenoids. Cells cylindrical and not narrowed at the posterior ends. Filaments straight. Cells usually twice as long as wide, and often with thick, laminate cell walls, *Rhizoclonium*

23c. Chloroplast a marginal band incompletely encircling the cell wall. Cells quadrangular, *Lothrix*

24a. Filaments not plantlike, not upright in water from sediment attachment, Go to 25

24b. Filaments plantlike, upright in water from sediment attachment, branches easily visible to the naked eye, Go to 27

25a. Apices of branches acutely pointed. Main axes usually less than 50 µm. Cells are thin walled and contain a single parietal chloroplast with one pyrenoid each. Thallus more delicate, *Stigeoclonium*

25b. Apices of branches rounded, each cell with a parietal and netlike chloroplast and many pyrenoids. Thallus substantial, often with a "brillo pad" feel. Main axes often greater than 50 µm, Go to 26

26a. Apices of branches rounded, filaments dichotomously or subdichotomously branched (sparse or profuse). Coarse in texture. Each cell with a parietal and netlike chloroplast and many pyrenoids, *Cladophora*

26b. Apices of branches rounded, filaments lateral or opposite and freely branched. Each cell with a netlike chloroplast and many pyrenoids. Very coarse in texture. Forms akinetes visible as thick-walled, dark cells (often swollen, and either terminal or intercalary), *Pithophora*

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27a. Thallus macroscopic, often highly encrusted to lightly encrusted, with a cortex of outer cells and with spine-cells, rigid, and symmetrical, *Chara*

27b. Thallus macroscopic, unencrusted to lightly encrusted, without a cortex of outer cells and without spine-cells, less rigid and less symmetrical, *Nitella*

REFERENCES

American Water Works Association (AWWA). 2002. *Identification of algae in water supplies.* CD-ROM. Denver, Colo.: AWWA.

American Water Works Association (AWWA). 2004. *Manual M7, Problem organisms in water: identification and treatment.* 3rd ed. Denver, Colo.: AWWA.

Bold, H., and M.C. Wynne. 1985. *Introduction to the algae.* Englewood Cliffs, N.J.: Prentice Hall.

Buchheim, M.A., M.A. McAuley, E.A. Simmer, E.C. Theriot, and R.L. Chapman. 1994. Multiple origins of colonial green flagellates from unicells: Evidence from molecular and organismal characters. *Mol. Phylog. Evol.*, 3:322–343.

Carpenter, S.R., J.A. Morrice, J.J. Elser, A. St. Amand, and N.A. MacKay. 1993. Phytoplanton community dynamics. In *The trophic cascade in lakes.* S.R. Carpenter and J.F Kitchell, eds. Cambridge, UK: Cambridge University Press.

Dobell, C. 1932. *Antony van Leewenhoek and his "little animals."* London: Staples Press.

Dodds, W.K., and D.A. Gudder. 1992. The ecology of *Cladophora. Jour. Phycol.*, 28:415–427.

Graham, L. 1996. Green algae to land plants: An evolutionary transition. *Jour. Plant Res.*, 109:241–251.

Graham, L.E. 1993. *The origin of land plants.* New York: John Wiley.

Graham, L.E., and L.E. Wilcox. 2000. *Algae.* Upper Saddle River, N.J.: Prentice Hall.

Hall, J.A., and N. Cox. 1995. Nutrient concentrations as predictors of nuisance *Hydrodicyton reticulatum* populations in New Zealand. *Jour. Aquat. Plant Manage.*, 33:68–74.

Happey-Wood, C.M. 1988. Ecology of freshwater planktonic green algae. In *Growth and reproductive strategies of freshwater phytoplankton.* C.D. Sandgren, ed. New York: Cambridge University Press.

Harris, V. 2004. *Cladophora* confounds coastal communities—Public perceptions and management dilemmas. In *Workshop proceedings:* Cladophora *research and management in the Great Lakes.* Special Report 2005-01. Milwaukee, Wis.: Great Lakes WATER Institute, University of Wisconsin.

Higgins, D. 2004. The contribution of *Dreissena* to the resurgence of *Cladophora* in eastern Lake Erie. In *Workshop proceedings:* Cladophora *research and management in the Great Lakes.* Special Report No. 2005-01. Milwaukee, Wis.: Great Lakes WATER Institute, University of Wisconsin.

Holdren, C., W. Jones, and J. Taggart. 2001. *Managing lakes and reservoirs.* North American Lake Management Society and Terrene Institute. In cooperation with the Office of Water Assessment and Watershed Protection, US Environmental Protection Agency (USEPA). Madison, Wis.: USEPA.

John, D.M., B.A. Whitton, and A.J. Brook. 2002. *The freshwater algal flora of the British Isles: An identification guide to freshwater and terrestrial algae.* New York: Cambridge University Press.

Kirk, J.T.O. 1983. *Light and photosynthesis in aquatic ecosystems.* Cambridge, UK: Cambridge University Press.

Lamberti, G.A., J.W. Feminella, and, C.M. Pringle. 2006. Primary producer-consumer interactions. In *Methods in stream ecology.* 2nd ed. F.R. Hauer and G Lamberti, eds. Amsterdam: Elsevier.

Lehman, J.T., and C.D. Sandgren. 1985. Species-specific rates of growth and grazing loss among freshwater algae. *Limnol. Oceanogr.*, 30:34–46.

Lembi, C. 2000. Relative tolerance of mat-forming algae to copper. *Jour. Aquat. Plant Manage.*, 38:68–70.

Lembi, C. 2003. Control of nuisance algae. In *Freshwater algae of North America, ecology and classification.* J.D. Wehr and R.G. Sheath, eds. New York: Academic Press.

Lowe, R.L., and G.D. LaLiberte. 2006. Benthic stream algae: Distribution and structure. In *Methods in stream ecology.* 2nd ed. F.R. Hauer and G. Lamberti, eds. Maryland Heights, Mo.: Elsevier.

Mallevialle, J., and I.H. Suffet. 1987. *Identification and treatment of tastes and odors in drinking water.* Denver, Colo.: Awwa Research Foundation.

Mishler B.D., L.A. Lewis, M.A. Buchheim, K.S. Renzaglia, D.J. Garbary, C.F. Delwiche, F.W. Zechman, T.S. Kantz, and R.L. Chapman. 1994. Phylogenetic relationships of the "green algae" and "bryophytes." *Ann. Mo. Bot. Garden*, 81:451–483.

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Munawar, M., and I.F. Munawar. 1976. A lakewide study of phytoplankton biomass and its species composition in Lake Erie, April–December, 1970. *Jour. Fish. Res. Board .Can.*, 33:581–600.

Neil, J.H. 1975. Ecology of the *Cladophora* niche. In *Cladophora* in the Great Lakes. H. Shear and D.E. Konasewich, eds. Windsor, Ont.: International Joint Commission Regional Office.

Prescott, G.W. 1982. *Algae of the Western Great Lakes area.* 2nd ed. Dubuque, Iowa: Wm. C. Brown.

Priya, A., P. Srinivasa Rao, Y. Vijaya, A. Subba Reddy, and A. Krishnaiah. 2007. Biosorption of chromium (VI), nickel (II), and copper (II) ions from aqueous solutions using *Pithophora* algae. *Toxicol. Environ. Chem.*, 89:421–442.

Reynolds, C.S. 1984. *The ecology of freshwater phytoplankton.* Cambridge, UK: Cambridge University Press.

Sandgren, C.D., P.M. Engevold, S. Neerhof, and T.J. Ehlinger. 2004. Nuisance *Cladophora* in urban streams: Habitats, seasonality, production, nutrient composition, heavy metals, foodweb bottleneck. In *Workshop proceedings:* Cladophora *research and management in the Great Lakes.* Special Report 2005-01. Milwaukee, Wis.: Great Lakes WATER Institute, University of Wisconsin.

Simons, J., M. Ohm, R. Daalder, P. Boers, and W. Rip. 1994. Restoration of Botshol (The Netherlands) by reduction of external nutrient load: Recovery of a characean community, dominated by *Chara connivens. Hydrobiologia*, 275-276:243–253.

Smith, G.M. 1950. *The freshwater algae of the United States.* New York: McGraw-Hill.

Sommer, U. 1989. The role of competition for resources in phytoplankton succession. In *Plankton ecology: Succession in plankton communities.* U. Sommer, ed. New York: Springer-Verlag.

Sournia, A. 1980. *Phytoplankton manual.* Paris: UNESCO Publishing.

Spencer, D.F., and C. Lembi. 1981. Factors regulating the spatial distribution of the filamentous alga *Pithophora oedogonia* (chlorophyceae) in an Indian lake. *Jour. Phycol.*, 17:168–173.

St. Amand, A.L. 1990. Mechanisms controlling metalimnetic communities and the importance of metalimnetic phytoplankton to whole lake primary productivity. Ph.D. diss., University of Notre Dame.

Stauffer, R. 2004. *Cladophora*: How is it handled? In *Workshop proceedings:* Cladophora *research and management in the Great Lakes.* Special Report 2005-01. Milwaukee, Wis.: Great Lakes WATER Institute, University of Wisconsin.

Sterner, R.W. 1989. The role of grazers in phytoplanton succession. In *Plankton ecology.* U. Sommer, ed. New York: Springer-Verlag.

Stutzman, P. 1995. Food quality of gelatinous colonial chlorophytes to the freshwater zooplankters *Daphnia pulicaria* and *Diaptomus oregonensis. Freshwater Biol.*, 34(1):149–153.

Turpin, D.H. 1988. Physiological mechanisms in phytoplankton resource competition. In *Growth and reproductive strategies of freshwater phytoplankton.* C.D. Sandgren, ed. New York: Cambridge University Press.

van den Berg, M.S., M. Scheffer, H. Coops, and J. Simmons. 1998. The role of characean algae in the management of eutrophic shallow lakes. *Jour. Phycol.*, 34:750–756.

van den Hoek, C., D.G. Mann, and H.M. Jahns. 1995. *Algae: An introduction to phycology.* Cambridge, UK: Cambridge University Press.

Van Donk, E. 1989. The role of fungal parasites in phytoplankton succession. In *Plankton ecology: Succession in plankton communities.* U. Sommer, ed. New York: Springer-Verlag.

Vasanth, K., S. Sivanesan, and V. Ramamurthi. 2005. Adsorption of malachite green onto *Pithophora* sp., a freshwater algae: Equilibrium and kinetic modelling. *Process Biochem.*, 40:2865–2872.

Wehr, J.D., and R.G. Sheath. 2003. *Freshwater algae of North America.* New York: Academic Press.

Wells, R.D.S., J.A. Hall, J.S. Clayton, P.D. Champion, G.W. Payne, and D.E. Hofstra. 1999. The rise and fall of water net (*Hydrodictyon reticulatum*) in New Zealand. *Jour. Aquat. Plant Manage.*, 37:49–55.

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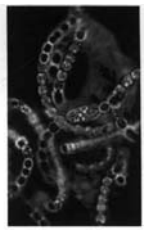

Chapter **7**

Euglenophyta

Grant G. Mitman

BIOLOGY

The etymology of *Euglena* (ModL < *eu-*, good, well + Gr *glene*, pupil of the eye) means "good eyeball," which probably refers to the prominent stigma or eyespot that makes this alga easy to identify (Figure 7-1). Euglenoids consist of 45 genera (Corliss 1984) and more than 1,000 species worldwide (Marin et al. 2003); roughly one-third of these are photosynthetic and considered algae rather than Protozoa. Phycologists and botanists place these organisms in the division Euglenophyta and class Euglenophyceae, with 6 orders: Eutreptiales, Euglenales, Euglenamorphales, Rhabdomomadales, Spenomonadales, and Heteronematales (Leedale 1967b). The discussion in this chapter will focus on the first three orders, which contain photosynthetic species (as well as heterotrophic species). Most euglenophytes are 20 μm–130 μm in size; they are grass green in color from their several chloroplasts with paramylon (β-1:3-linked glucan) (Leedale 1967a) as a storage product that may or may not be associated with pyrenoids. Euglenids are oval to spindle-shaped and usually motile by metaboly (euglenoid movement) or anterior flagella (0–3 emergent), with a bright red to orange stigma or eyespot, and a striated cell surface (pellicle). Although there are some marine and brackish water species, this chapter will focus on the freshwater species, which can easily be found ubiquitously in waters from low to high pH and oligotrophic to eutrophic conditions worldwide. The life cycles of all known species is largely asexual by longitudinal mitosis with a few reports of autogamous meiosis (Wolowski 2002).

Euglenoid morphology is generally spindle-shaped, which makes them easily recognizable. However, there is significant variation around this theme. Spindle-shaped *Euglena* may be straight, twisted, triradiate, circular, or laterally compressed. A number of species can also change their shape by metaboly (euglenoid movement), which can also help them move along substrates by undulating contractions (*Euglena, Trachelomonas,* and *Colacium*).

Euglenoid organisms do not have true cell walls but the plasma membrane is often supported by a proteinaceous pellicle of varying thickness and can be flexible or rigid. In addition, two genera, *Trachelomonas* and *Strombomonas*, have an additional mucilaginous covering known as the lorica. The lorica can be dark brown due to being heavily mineralized by iron and manganese and may be spiny. Finally, one species,

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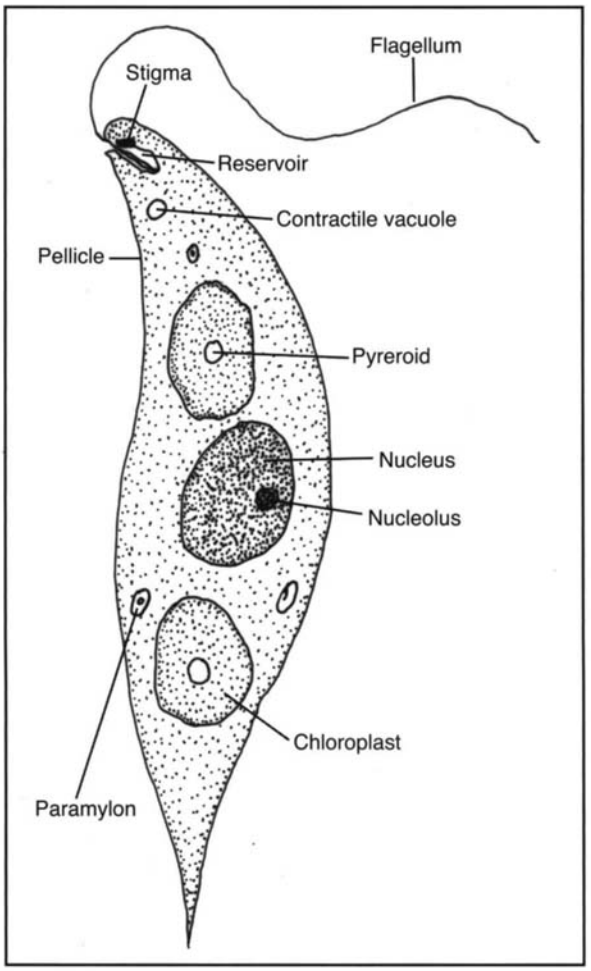

Figure 7-1 Labeled *Euglena*

Colacium, produces mucilage at the canal end, resulting in a dichotomously stalked form.

TAXONOMY

There are three orders of euglenophytes that have photosynthetic representatives: the Eutreptiales, Euglenales, and Euglenamorphales. The Eutreptiales have two emergent flagella of equal length and also display metaboly to assist in their movement. In contrast, the Euglenales only have one of their two flagella emergent (*Euglena mutabilis*—none emergent) with some species displaying metaboly. Finally, the Euglenamorphales consist of endozoic genera with three or more flagella. For a detailed description of species, see Rosowski 2003 for North America and Wolowski 2002 for the British Isles. For a description of the euglenozoa, see Leedale and Vickerman 2000.

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KEY TO FRESHWATER GENERA OF PHOTOSYNTHETIC EUGLENOIDS*

1a Cells attached to aquatic arthropod cuticles (mostly microcrustacea), singly or in palmelloid colonies; sometimes on branched, mucilaginous stalks; become metabolic, *Colacium*

1b. Cells motile and solitary; or if in a palmella stage, not on arthropod cuticles, 2

2a. Cells with a lorica (case, envelope), often dark-brown colored, 3

2b. Motile cells without a lorica (naked) and never developing one, 4

3a. Lorica attached posteriorly, with anterior opening the width of the cell, *Ascoglena*

3b. Loricate cells unattached and motile; anterior lorica opening <¼ width of lorica, 5

4a. Motile cells with one emergent flagellum, 6

4b. Motile cells with two or more emergent flagella, 7

5a. Lorica light to dark reddish brown, rigid and brittle when highly pigmented; cell and lorica ovoid or sometimes spherical; collar, if present, abrupt, *Trachelomonas*

5b. Lorica clear or yellowish, flexible, typically tapering to both apices; collar base tapered, not abrupt, *Strombomonas*

6a. Motile cells with a deep ventral sulcus when viewed apically, *Cryptoglena*

6b. Motile cells without a deep sulcus (apex-to-apex longitudinal groove), 8

7a. Motile cells with two emergent flagella of equal length no stage in tadpole, *Eutreptia*

7b. Cells with three emergent flagella; palmelloid in tadpole rectum, *Euglenamorpha*

8a. Cells may change shape; canal opens subapically, *Euglena*

8b. Cells with a firm pellicle (surface usually striated), 9

9a. Cells circular in apical view; or if otherwise, pellicle firm, 10

9b. Cells laterally flattened, leaf-shaped, and perhaps twisted as well, *Phacus*

10a. Subapical canal opening, *Euglena*

10b. Apical canal opening; cells transversely circular, not flattened, *Lepocinclis*

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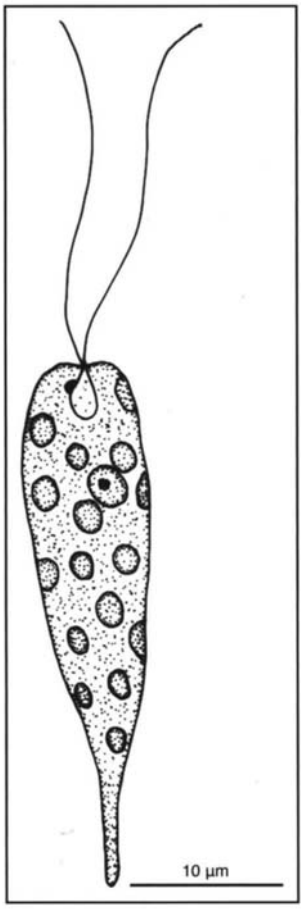

Figure 7-2 *Eutreptia viridis*

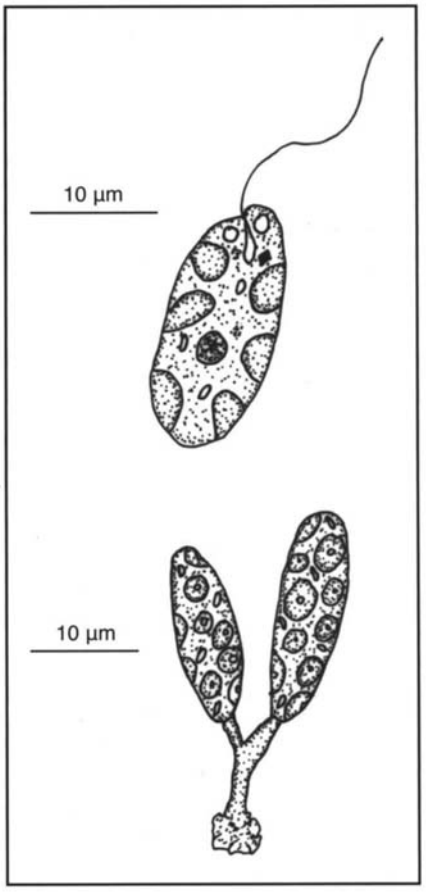

Figure 7-3 *Colacium vesiculosum*

Order: Eutreptiales

Motile Euglenids with two emergent flagella of even to uneven length displaying metaboly.

Genus: *Eutreptia* (Perty 1852) (Figure 7-2). This is the only photosynthetic species described for North America (Rosowski 2003). *Eutreptia* is a very motile species with its two emergent flagella and active metaboly. It occurs as a spindle-shaped unicell with a slightly striated pellicle and large eyespot. Chloroplasts are discoid without pyrenoids, but often have small paramylon bodies. Resting cysts have been observed. *Eutreptia viridis* Perty 1852 is a spindle-shaped species, has a noticeable cauda or tail, and is 13–25 µm wide and 49–66 µm long (Wolowski 2002). It has been reported from North Carolina in the United States (Whitford and Schumacher 1969).

Order: Euglenales

Motile Euglenids with one of the two flagella emergent (none emergent *Euglena mutabilis,* three emergent *Euglenamorpha hegneri*) from the reservoir and canal. Cells are oval to elongate or cylindrical, with some species displaying metaboly.

Genus: *Colacium* (Ehrenberg 1838) (Figure 7-3). This species is easily characterized because it often occurs as a sessile dendritic dichotomously branched colony connected by mucilaginous stalks; although cells are occasionally free-swimming. Cell shape is oval to ellipsoidal with an eyespot, single emergent flagellum, and lightly

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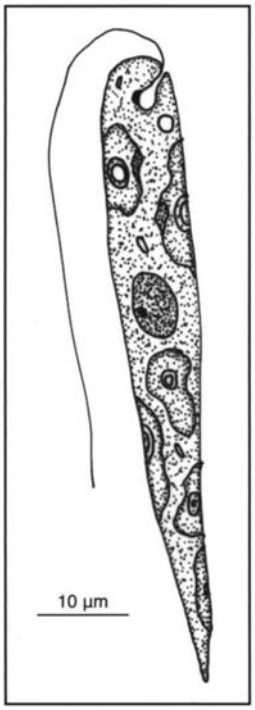

Figure 7-4 *Euglena caudata*

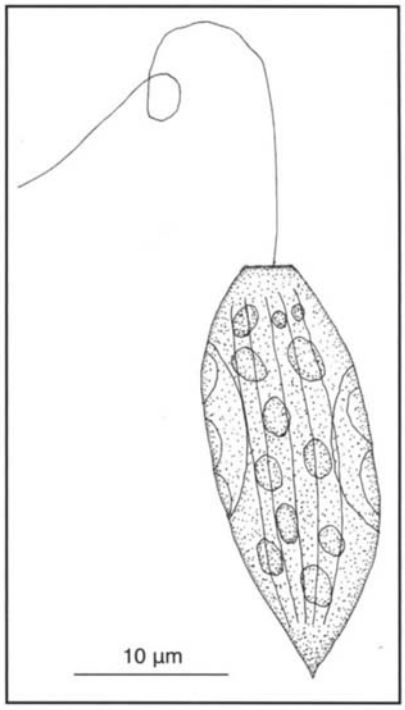

Figure 7-5 *Lepocinclis steinii*

striated pellicle. Chloroplasts are numerous and discoid, lacking pyrenoids, but with small oval paramylon bodies. Cells contain one to eight nuclei.

Genus: *Euglena* (Ehrenberg 1838) (Figure 7-4). This is the most frequently encountered and ubiquitous genus for which the division is named. Genera are motile by a single emergent flagellum (except *E. mutabilis*) and differing metaboly depending on species. Unicells are ovoid to spindle-shaped and sometimes twisted, often terminating to posterior attenuated cauda and an anteriorly located eyespot. Chloroplasts are numerous, discoid to bandlike, sometimes with pyrenoids and usually with numerous paramylon bodies. Striated pellicle is semirigid to rigid, allowing for varying degrees of metaboly. *Euglena* is found from oligotrophic to eutropic conditions in acidic to basic waters (pH 0.9–8.9).

Genus: *Lepocinclis* (Perty 1849) (Figure 7-5). This is a solitary, radially symmetrical (nonflattened) euglenophyte, oval to spindle-shaped, with a posterior caudus of varying length bearing a single emergent anterior flagellum and anterior stigma. This genus has a rigid pellicle (no metaboly) with spirally arranged striae. Numerous chloroplasts are parietal and lack pyrenoids; however two to four circular shaped paramylon bodies are often present. Cysts and palmelloid stages reported.

Genus: *Phacus* (Dujardin 1841) (Figure 7-6). This is another species with a rigid pellicle (no metaboly). However, unlike *Lepocinclis*, this solitary species with a single emergent flagellum is flattened and often twisted in shape, ovoid to fusiform, with distinctive spiral ornamentation on the pellicle. Chloroplasts are numerous and discoid with or without pyrenoids depending on the species. Ring-shaped paramylon bodies may be present. The stigma my be present or absent; cysts have been reported.

Genus: *Strombomonas* (Deflandre 1930) (Figure 7-7). This has the general *Euglena* form, but it is a solitary, loricate species appearing as a cell within a cell, with an indistinct anterior collar. The lorica is yellow to light brown, nonornamented, unmineralized, and pliable, and can change shape during metaboly. There is an anterior stigma

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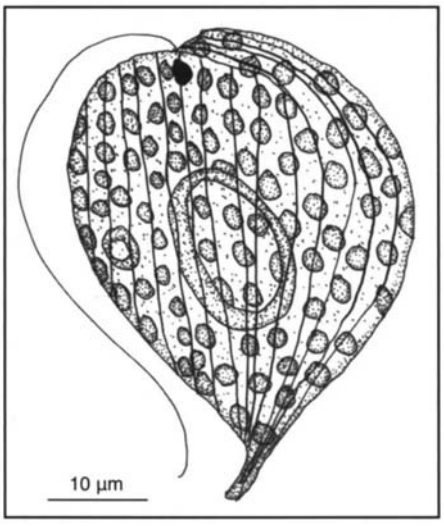

Figure 7-6 *Phacus pleuronectes*

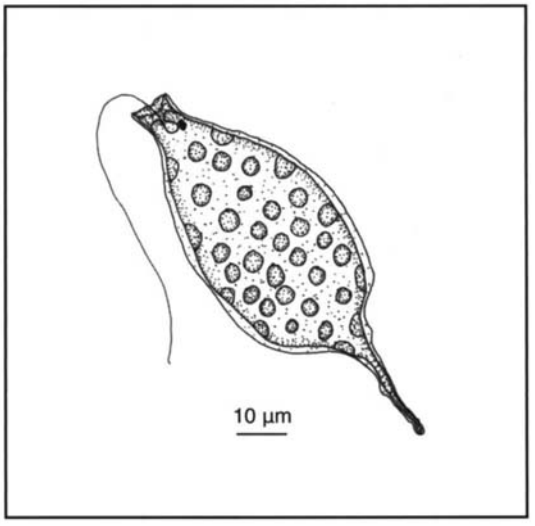

Figure 7-7 *Strombomonas fluviatilis*

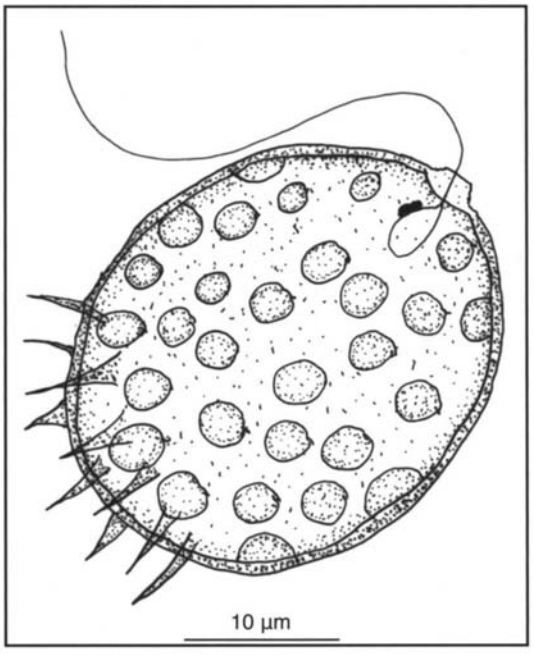

Figure 7-8 *Trachelomonas armata*

and a single emergent, subabically inserted flagellum. Chloroplasts are discoid, and paramylon bodies may be used to distinguish different species. Cysts have not been reported.

Genus: *Trachelomonas* (Ehrenberg 1838) (Figure 7-8). This is a distinctly loricate species with the general *Euglena* form. The lorica of this species is rigid and often mineralized by iron and other heavy metals, is ornamented, and has a distinct anterior collar. Although metaboly of the inner cell can be observed, the outer lorica maintains its shape. This solitary species is motile by a single emergent flagellum that emerges from the collar. Chloroplasts are often small and discoid, with paramylon bodies and usually without pyrenoids. Cysts have been reported.

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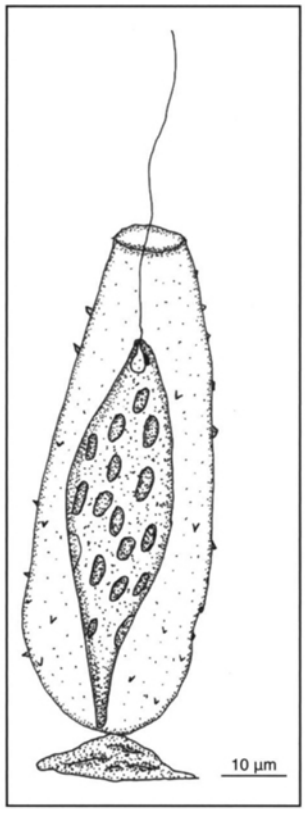

Figure 7-9 *Ascoglena vaginicola*

Genus: *Ascoglena* (Stein 1878) (Figure 7-9). This is a rarely observed sessile euglenid flagellate, solitary or attached, located within a cup-shaped, yellow-brown to reddish ferric embedded lorica open at the anterior end. The cell is *Euglena*-like in shape and is supported by a short stalk. It has numerous discoid chloroplasts, with or without pyrenoids, a single emergent flagellum, an eyespot, and metabolic movement.

Genus: *Cryptoglena* (Ehrenberg 1838) (Figure 7-10). *Cryptoglena* is a dorsoventrally flattened, oval, solitary, free-swimming flagellate with a single emergent flagellum arising from a slight indentation in the anterior end of the cell. There is a small stigma. The pellicle is rigid (no metaboly) with a rounded anterior and dully pointed posterior. There is an obvious longitudinal furrow (sulcus). It is described as having a single shieldlike chloroplast, which often has been described as two separate chloroplasts but is really just thinner in the middle (Rosowski 2003) without pyrenoids.

Order: Euglenamorpha

This order only contains the monotypic genus *Euglenamorpha* in North America recorded from the digestive tract of *Rana* tadpoles.

Genus: *Euglenamorpha* (Wenrich 1924) (Figure 7-11). This is morphologically similar to *Euglena* but is immediately distinguished by its three emergent flagella. Green, cigar-shaped cells (50–10 µm) with discoid parietal chloroplasts lacking pyrenoids but with paramylon bodies are readily observed. This species is found exclusively in the rectum of the tadpoles *Rana pipiens* and *R. clamitans* (Wenrich 1923).

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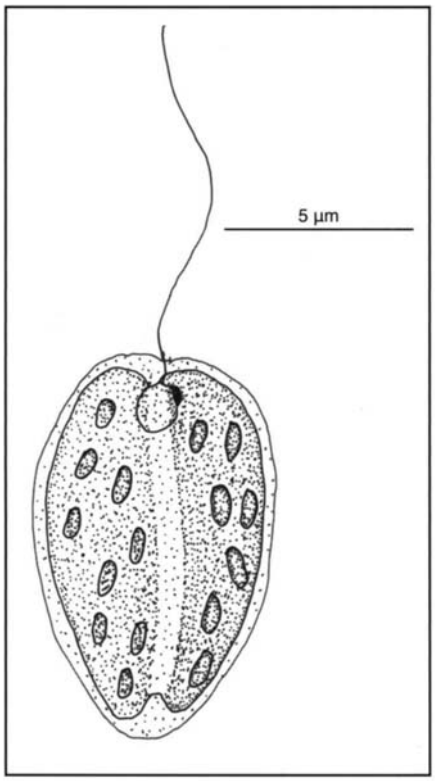

Figure 7-10 *Cryptoglena pigra*

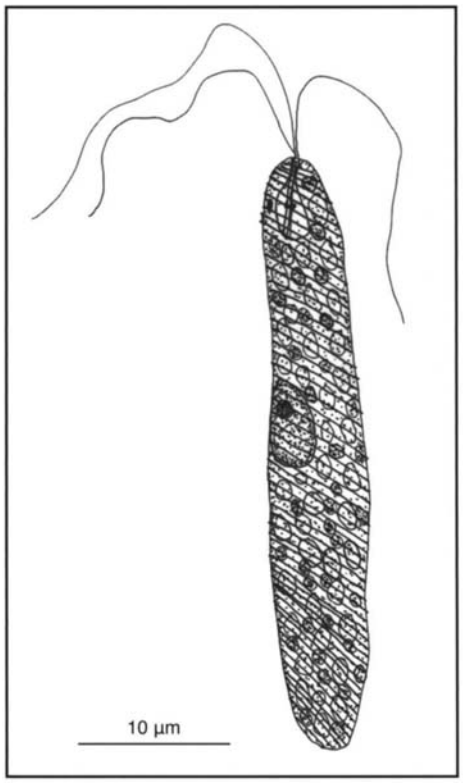

Figure 7-11 *Euglenamorpha hegneri*

REFERENCES

Corliss, J.O. 1984. The kingdom Protista and its 45 phyla. *Biosystems*, 17(2): 87–126.

Deflandre, G. 1930. *Strombomonas*, nouveaux genre d'Euglénacées (*Trachelomonas* Ehrbg. *pro parte*). *Arch. für Protisten.*, 69:551–614.

Dujardin, F. 1841. *Histoire naturelle des zoophytes infusoires*. Paris: Roret.

Ehrenberg, C.G. 1838. *Die Infusionsthierchen als volkommene Organismen*. Leipzig: Verlag von Leopold Voss.

Leedale, G.F. 1967a. Euglenida/euglenophyta. *Ann. Rev. Microbiol.*, 21:31–48.

Leedale, G.F. 1967b. *Euglenoid flagellates*. Englewood Cliffs, N.J.: Prentice Hall.

Leedale, G.F., and K. Vickerman. 2000. Phylum euglenozoa. In *The illustrated guide to the protozoa*. 2nd ed. J.J. Lee, G.F. Leedale, and P. Bradbury, eds. Lawrence, Kan.: Allen Press.

Marin, B., A. Palm, M. Klingberg, and M. Melkonian. 2003. Phylogeny and taxonomic revision of plastid-containing euglenophytes based on SSU rDNA sequence comparisons and synapomorphic signatures in the SSU rRNA secondary structure. *Protist*, 154(1):99–145.

Perty, M. 1849. Über verticale Verbreitung mikroskopischer Lebensformen. *Mitteilungen der Naturforschenden Gesellschaft in Bern*, 146-149:17–45.

Perty, M. 1852. *Zur Kenntnis kleinster Lebensformen: nach Bau, Funktionen, Systematik, mit Spezialverzeichniss der in der Schweiz beobachteten*. Bern: Verlag von Jent und Reinert.

Rosowski, J.R. 2003. Photosynthetic euglenoids. In *Freshwater algae of North America ecology and classification*. J.D. Wehr and R.G. Sheath, eds. San Diego, Calif.: Academic Press.

Stein, R.R. 1878. *Der Organismus der Infusionsthiere. Part 3: Der Organismus der Flagellaten*. Leipzig: Hälfte.

Wenrich, D.H. 1923. Variations in *Euglenamorpha hegneri* n.g., n.sp., from the intestine of tadpoles. *Anatom. Rec.*, 24:370–371.

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Wenrich, D.H. 1924. Studies on *Euglenamorpha hegneri* n.g., n.sp., a euglenoid flagellate found in tadpoles. *Biolog. Bull.*, 47:149–1741.

Whitford, L.A., and G. Shumacher. 1969. *A manual of freshwater algae in North Carolina.* North Carolina Agricultural Station Technical Bulletin 188.

Wolowski, K. 2002. Phylum euglenophyta. In *The freshwater algae of the British Isles: An identification guide to freshwater and terrestrial algae.* D.M. John, B.A. Whitton, and A.J. Brook, eds. Cambridge, UK: Cambridge University Press.

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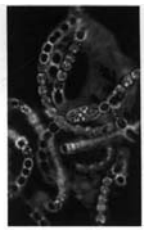

Chapter 8

Dinophyta

Susan Carty

Most freshwater dinoflagellates are unicells that can be recognized by the swimming pattern, golden color, and indented waist (cingulum). Of course, some dinoflagellates are immobile, some are not golden, and some lack a cingulum, but living cells will usually have at least one of these characteristics.

BIOLOGY

Dinoflagellates are a group of protists that are freshwater and marine; photosynthetic, heterotrophic, or both; have some unique organelles; and are important components of the plankton. Some estuarine species are responsible for red tides and cause paralytic shellfish poisoning (PSP), ciguatera, and fish kills (Hallegraeff 1993). Dinoflagellates can be distinguished by a suite of characters including a large nucleus with permanently condensed chromosomes, dissimilar flagella resulting in a distinctive swimming pattern, and yellow to golden-brown accessory pigments.

Dinoflagellates are described as armored (thecate) or naked depending on the presence or absence of a cellulose wall, respectively. In either case, at some time in the life cycle, the cell is partitioned into an upper half and lower half by a transverse groove called the cingulum. The cingulum houses a ribbonlike tinsel flagellum. A vertical groove, called the sulcus, directs a narrower, whiplash flagellum posteriorly. The sulcus is on the ventral face of the cell, and so a dinoflagellate has a ventral and dorsal surface, apical and antapical views, and a right and left side (Figure 8-1). The whiplash flagellum propels the cell forward, and the ribbonlike flagellum causes the cell to spin, producing a distinctive spiral swimming pattern.

Photosynthetic dinoflagellates may have their own yellow to golden-brown chloroplasts, or may use plastids from other organisms (kleptoplastids) that give them a blue-green or other color. Heterotrophic dinoflagellates may be colorless, have lightly pigmented pink cytoplasm, or retain bright red, orange, or yellow bodies in the cytoplasm (Figure 8-2). Parasitic dinoflagellates may be found attached to filamentous green algae or fish.

Internally, dinoflagellates have a large nucleus with permanently condensed chromosomes that looks like a fingerprint (can be seen with a light microscope) (Figure 8-3), some species have a red eyespot in the sulcus, and all store starch (turn black in the presence of iodine).

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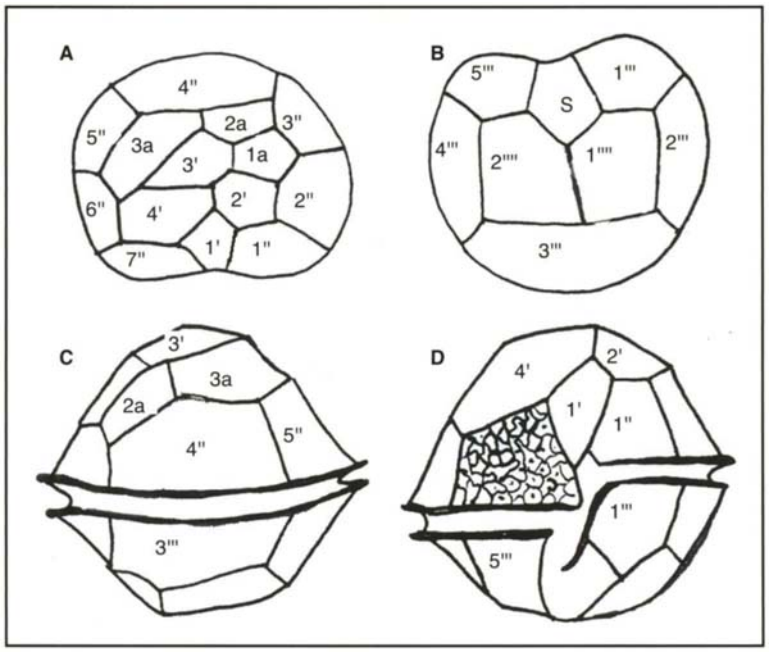

Figure 8-1 Outline of armored dinoflagellate showing (A) apical view, (B) antapical, (C) dorsal, (D) ventral with cingulum and sulcus; plates annotated

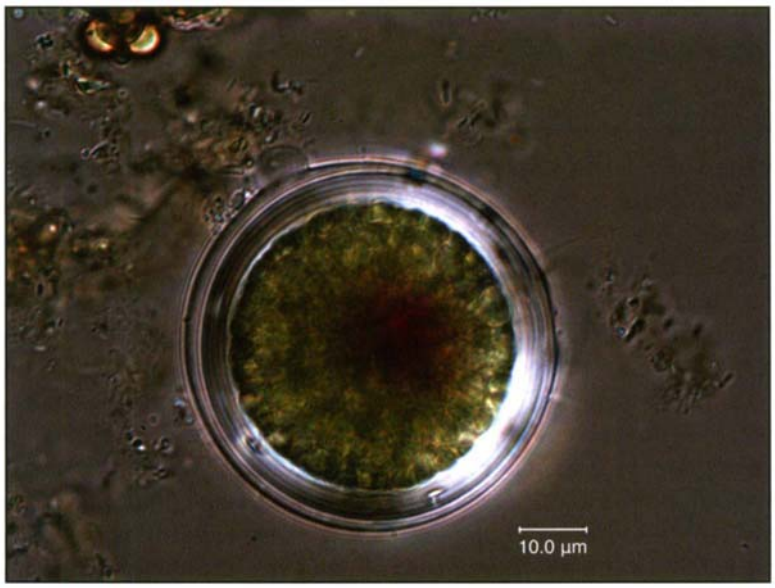

Figure 8-2 *Gloeodinium montanum* showing a red colored body inside the cell

The dinoflagellate life cycle includes an assimilative, vegetatively reproducing stage that alternates with a resting cyst stage that may be the result of sexual reproduction. The cysts remain viable in the sediments and serve as seed stock for future years. The annual cycle of *Ceratium hirundinella* begins with excystment in temperate lakes as temperatures increase in the spring (Heaney et al. 1983, Pollingher et al. 1993), continues with vegetative reproduction, and ends with encystment as nutrients are depleted (Pollingher 1987).

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Figure 8-3 *Ceratium hirundinella* with large, centrally located nucleus

TAXONOMY

Dinoflagellates are described as thecate/armored or athecate/naked depending on the presence of a cellulose wall in the former. When a cell wall is present, it is divided into plates and the number and arrangement of plates determines genus and species. Plates are usually arranged in concentric series and are named and annotated in relationship to their proximity to the cingulum (transverse groove) (Figure 8-1). Plates above the cingulum are precingular (″); below are postcingular (‴). Plates at the cell apex are apical (′); those at the posterior are antapical (⁗). Plates between apical and precingular are apical intercalary (a); plates between antapex and postcingular are posterior intercalary (p). Plates are numbered counterclockwise from the sulcus (Figure 8-1). All thecate dinoflagellates have a plate pattern; for *Ceratium* it is 4′, 5″, 5‴, 2⁗, the numbers indicating the number of plates in each series. In addition to the four main series of plates, plates in the cingulum (C) and sulcus (S) may be included in a formula; *Ceratium* is 4′, 5″, 5C, 4S, 5‴, 2⁗. Plate shape, symmetry, and ornamentation are also part of the species descriptions. For thecate dinoflagellates, the plate pattern is definitive for species identification. Other characteristics such as pigmentation or its lack; presence/absence of an eyespot; number, shape and placement of spines; and presence/absence of an apical pore may aid identification.

Lacking a cellulose wall, the naked dinoflagellates are identified based on characteristics best (or only) seen in living cells. A typical naked dinoflagellate, such as *Gymnodinium*, is identified based on its overall shape, features of the cingulum and sulcus, color, and presence/absence of an eyespot.

Several genera and species of dinoflagellate are documented as producing blooms. Blooms are large populations of a single species of alga. The best known is *Ceratium*. Almost all references to *Ceratium* blooms cite *C. hirundinella* as the responsible species but cells should be examined to confirm identification. *C. hirundinella* (Figure 8-4) and most of its forms have three posterior horns; *C. rhomvoides* (previously called *C. hirundinella* form *gracile*) has two posterior horns (Figure 8-5) and can be dominant (Gligora et al. 2003).

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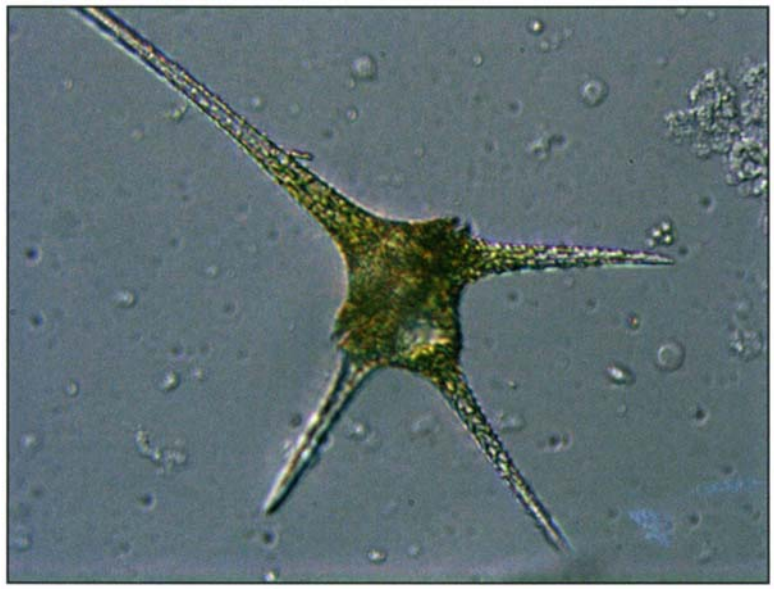

Figure 8-4 *Ceratium hirundinella*: note three posterior horns, golden color

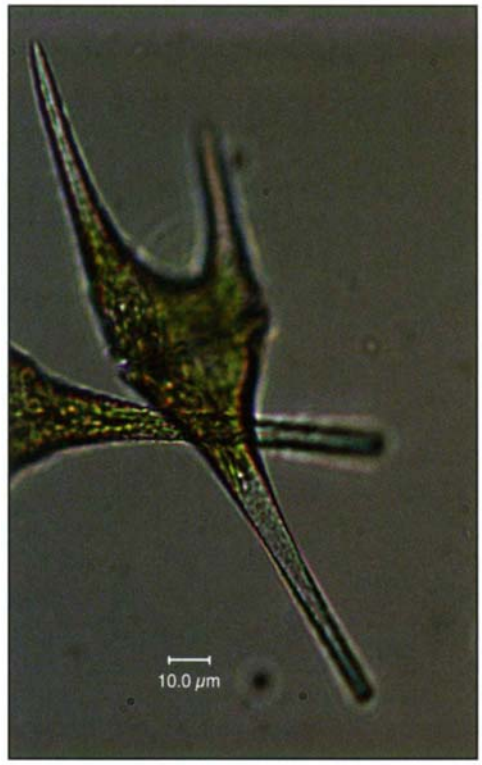

Figure 8-5 *Ceratium rhomvoides*: note two posterior horns

Peridinium species (4′, 3a, 7″, 5‴, 2⁗) are sometimes responsible for blooms in reservoirs and may cause odor problems. *Peridinium bipes* (Japan, Yoshikawa et al. 1997; Taiwan, Wu and Chou 1998), *P. gatunense* (Israel, Lindström 1991), *P. inconspicuum* (USA, Koryak 1978), *P. limbatum* (Canada, Yan and Stokes 1978), *P. lomnickii* (England, Cranwell et al. 1985), *P. pusillum* (USA, Stoneburner and Smock 1980), and *P. willei* (USA, Stewart and Blinn 1976) have all been reported as bloom-producing species. *Peridiniopsis* species (3–5′, 0 or 1a, 6–7″, 5‴, 2⁗) such as *Per. cunningtonii*

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Figure 8-6 *Peridinium gatunense*: note golden color and Saturn appearance

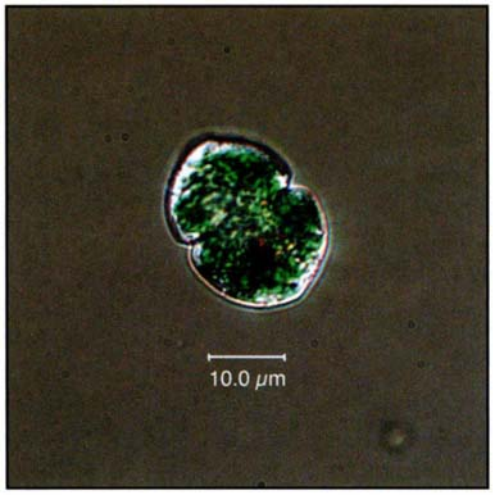

Figure 8-7 *Gymnodinium aeruginosum*: note blue-green color, noticeable cingulum, and red eyespot

(Japan, Sako et al. 1984) and *Per. penardii* (USA, Horne et al. 1971) have been reported as producing 5,000 cells/mL (Horne et al. 1971). *Woloszynskia reticulata* was reported in bloom condition from a lake in Oklahoma in August (Pfiester et al. 1980).

In my own experience of sampling temperate lakes across the United States for the past 30 years, I have seen blooms in reservoirs of *C. hirundinella*, *P. gatunense* (recognized by its likeness to the planet Saturn, a round cell with the cingular edges extended to look like rings) (Figure 8-6), and *P. willei* (longer than wide with crests at apex and antapex). I have seen a bloom of *Gymnodinium aeruginosum* (bluegreen) (Figure 8-7) in a reservoir north of Columbus, Ohio. *Peridiniopsis polonicum* can also be found in large numbers in some lakes; it can be recognized as a large cell (33–54 µm long), strongly dorsoventrally compressed, with a single, stout spine at the antapex (Figure 8-8). This species is important because it is the only known toxin-producing freshwater dinoflagellate.

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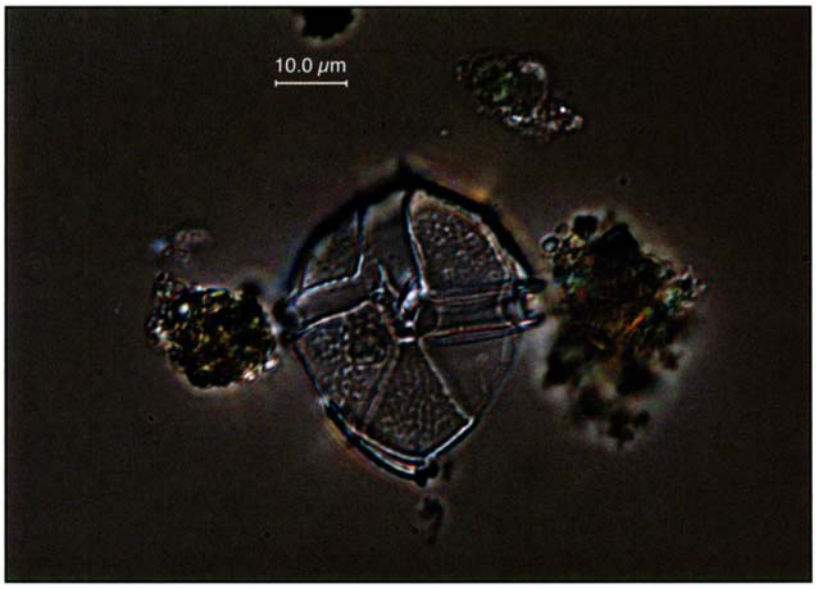

Figure 8-8 *Peridiniopsis polonicum*: note single antapical spine

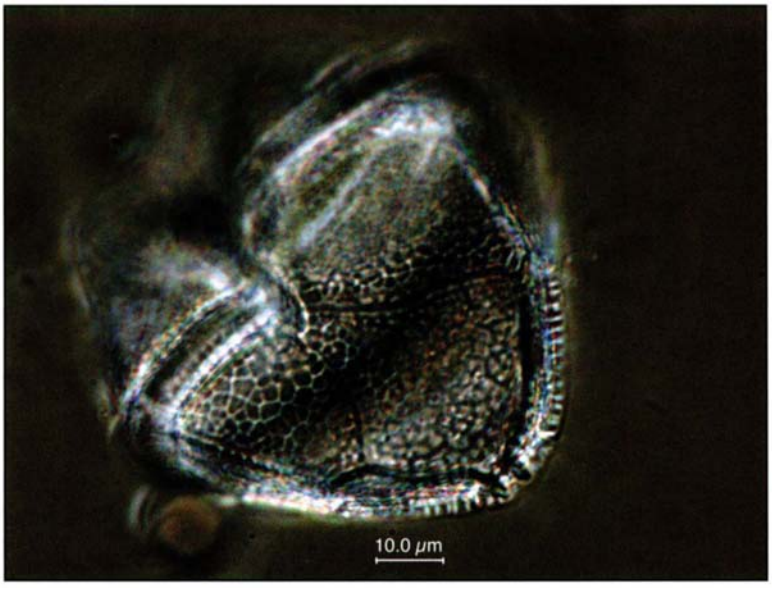

Figure 8-9 *Peridinium bipes*: empty theca showing plates, large 1´ plate, and apical pore

ECOLOGY

Occurrence

Although dinoflagellates can be found in all types of freshwater environments including lakes, ponds, ditches, fish farm ponds, and moving water, they are primarily of concern when they produce blooms in drinking water reservoirs. Dinoflagellates may be present in freshwater year-round but are most likely to bloom during summer months in temperate regions. This typical summer pattern for *C. hirundinella* may be reversed in tropical lakes with summer/high temperature encystment (Pollinger and Hickel 1991) and winter maximum in southern temperate lakes (Spain, Pérez-Martínez and Sánchez-Castillo 2001, 2002).

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Distribution

The most recognized and well-known freshwater dinoflagellate, *C. hirundinella*, has been reported throughout North America, Europe, Africa, and Asia (Carty 2003) and is probably present in most large bodies of water. Although dinoflagellates are part of the plankton and have little resistance to currents (APHA et al. 2005), their flagella allow them to move vertically in a lake, rising and sinking diurnally. *Ceratium* migrates into surface waters during the day (though it avoids a strongly illuminated surface) and sinks at night (Heaney and Talling 1980). Large numbers may be found at depth (60–70 m) in clear lakes during the summer (Moore 1981), correlated with a deep chlorophyll maximum (Echevarria and Rodriquez 1994, Gálvez et al. 1988). In addition to vertical distribution, there is also variable horizontal distribution. In reservoirs with inflows from rivers, *C. hirundinella* populations increased from where the river entered (with high N and P) to the dam (Kawabata and Kagawa 1988). Another example is the range of 67–5,360 cells/mL of *P. bipes* across a reservoir in Japan (Hirabayashi et al. 2007).

Growth Conditions

The term *red tide* is usually used when blooms of estuarine, toxin-producing dinoflagellates discolor the water and is a warning to avoid eating shellfish, which concentrate the toxins (see Okaichi et al. 1989). The term has also been used to describe freshwater dinoflagellate blooms. As with all organisms, dinoflagellate populations are controlled by top-down influences such as predation (by fish, Hambright et al. 2002; by rotifers, Stelzer 1998; by *Chaoborus*, Shei et al. 1988) and disease, and bottom-up influences such as nutrient levels (nitrogen and phosphorus, Nakano et al. 1999, James et al. 1992). Motility allows *C. hirundinella* to migrate into P-rich areas (the hypolimnion before anoxia and subsurface stream inflows), which enhances production (James et al. 1992). Environmental factors also contribute. Wind can cause transport and concentration of cells at one end of a reservoir (Heaney and Talling 1980). Rainfall can contribute dissolved inorganic nitrogen to fuel a bloom (Nakano et al. 1999). Day length, water temperature, and stratification also influence the formation of blooms, as do hydrological features related to water inflow and discharge of large, multiuse reservoirs (Pérez-Martínez and Sánchez-Castillo 2002).

Significance

Dinoflagellates may be important members of the phytoplankton in water supply reservoirs. *Peridinium* and *Ceratium* have classically been listed as taste and odor algae (APHA et al. 2005). Blooms of *Peridinium* may clog filters and produce offensive taste and odor in water supplies (Park and Hayashi 1993).

Marine and estuarine dinoflagellates can be toxic, producing a suite of neuro-, entero-, and ichthyotoxins. Fortunately, the only freshwater dinoflagellate with documented toxin production is *Per. polonicum* (Figure 8-8). Massive fish kills associated with a bloom of *Per. polonicum* in Japan led to isolation of glenodinine, a water-soluble alkaloid with a sulfhydryl radical (Hashimoto et al. 1968). Toxins from *Per. polonicum* isolated from blooms were shown to be toxic to killifish (Oshima et al. 1989), and this species was responsible for fish death at a Spanish trout farm that received discharge water from a hydroelectric facility (Roset et al. 2002). *P. bipes* (Figure 8-9) showed no toxicity for fish (Oshima et al. 1989), though it was algicidal toward *Microcystis aeruginosa* (Wu et al. 1998). Dinoflagellate blooms may be associated with fish kills if high numbers induce anoxia at night or water conditions lead to formation of ammonia (Van Ginkel et al. 2001).

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KEY TO GENERA*

1a. Cell with firm outer wall, sharply demarcated cingulum, 2

1b. Cell without outer wall, easily deformed, *Gymnodinium*

2a. Cell with 3–4 slim, elongated extensions, *Ceratium*

2b. Cell round to ovoid, plates more or less visible, 3

3a. Cell round, plates thin, *Woloszynskia* (and others)

3b. Cell round or angular, may have spines, crests, 4

4a. Plates in 4′, 3a, 7″, 5‴, 2⁗ pattern, *Peridinium*

4b. Plates in 3-5′, 0 or 1a, 6–7″, 5‴, 2⁗ pattern, *Peridiniopsis*

REFERENCES

American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF). 2005. *Standard methods for the examination of water and wastewater.* 21st ed. Washington, D.C.: APHA.

Carty, S. 2003. Dinoflagellates. In *Freshwater algae of North America: Ecology and classification.* J.D. Wehr, and R.G. Sheath, eds. Burlington, Mass.: Academic Press.

Cranwell, P.A., N. Robinson, and G. Eglinton. 1985. Esterified lipids of the freshwater dinoflagellate *Peridinium lomnickii. Lipids*, 20:645–651.

Echevarria, F., and J. Rodriguez. 1994. The size structure of plankton during a deep bloom in a stratified reservoir. *Hydrobiologia*, 284:113–124.

Gálvez, J.A., F.X. Niell, and J. Lucena. 1988. Description and mechanism of formation of a deep chlorophyll maximum due to *Ceratium hirundinella* (O.F. Müller) Bergh. *Arch. Hydrobiol.*, 112:143–155.

Gligora, M., A. Plenkovic, and I. Ternjej. 2003. Seasonal distribution and morphological changes of *Ceratium hirundinella* in two Mediterranean shallow lakes. *Hydrobiologia*, 506-509:213–220.

Hallegraeff, G.M. 1993. A review of harmful algal blooms and their apparent global increase. *Phycologia*, 32(2):79–99.

Hambright, K.D., S.C. Blumenshine, and J. Shapiro. 2002. Can filter-feeding fishes improve water quality in lakes? *Freshwater Biol.*, 47:1173–1182.

Hashimoto, Y., T. Okaichi, L.D. Dang, and T. Noguchi. 1968. Glenodine, an ichthyotoxic substance produced by a dinoflagellate, *Peridinium polonicum. Bull. Jpn. Soc. Sci. Fish.*, 34(6):528–534.

Heaney, S.I., and J.F. Talling. 1980. Dynamic aspects of dinoflagellate distribution patterns in a small productive lake. *Jour. Ecol.*, 68:75–94.

Heaney, S.I., D.V. Chapman, and H.R. Morison. 1983. The role of the cyst stage in the seasonal growth of the dinoflagellate *Ceratium hirundinella* within a small productive lake. *Br. Phycol. Jour.*, 18:47–59.

Hirabayashi, K., K. Yoshizawa, N. Yoshida, K. Ariizumi, and F. Kazama. 2007. Long-term dynamics of freshwater red tide in shallow lake in central Japan. *Environ. Health Prevent. Med.*, 12:33–39.

Horne, A., P. Javornicky, and C. Goldman. 1971. A freshwater "red tide" of Clear Lake, California. *Limnol. Oceanogr.*, 16:684–689.

James, W.F., W.D. Taylor, and J.W. Barko. 1992. Production and vertical migration of *Ceratium hirundinella* in relation to phosphorus availability in Eau Galle Reservoir, Wisconsin. *Can. Jour. Fish. Aquat. Sci.*, 49:694–700.

Kawabata, Z., and H. Kagawa. 1988. Distribution pattern of the dinoflagellate *Ceratium hirundinella* (O.F. Müller) Bergh in a reservoir. *Hydrobiologia*, 169:319–325.

Koryak, M. 1978. The occurrence of *Peridinium inconspicuum* Lemmermann (Dinophyceae) in minerally acid waters of the Upper Ohio River basin. *Proc. Acad. Nat. Sci. Philadelphia*, 130:21–25.

Lindström, K. 1991. Nutrient requirements of the dinoflagellate *Peridinium gatunense. Jour. Phycol.*, 27:207–219.

* A more complete key to genera can be found in Carty (2003). Books including keys to species include Popovský and Pfiester (1990) and Starmach (1974). Author is currently preparing a key to species in North America (Carty, in prep.)

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Moore, J.W. 1981. Seasonal abundance of *Ceratium hirundinella* (O.F. Müller) Schrank in lakes of different trophy. *Arch. Hydrobiol.*, 92:535–548.

Nakano, S., T. Nakajima, K. Hayakawa, M. Kumagai, and C. Jiao. 1999. Blooms of the dinoflagellate *Ceratium hiundinella* in large enclosures placed in Lake Biwa. *Jpn. Jour. Limnol.*, 60(4):495–505.

Okaichi, T., D.H. Anderson, and T. Nemoto (eds). 1989. Red tides: Biology environmental science and toxicology. In *Proceedings of the first international symposium on red tides.* New York: Elsevier Press.

Oshima, Y., H. Minami, Y. Takano, and T. Yasumoto. 1989. Ichthyotoxins in a freshwater dinoflagellate *Peridinium polonicum.* In *Proceedings of the first international symposium on red tides.* T. Okaichi, D.H. Anderson, and T. Nemoto, eds. New York: Elsevier Press.

Park, H-D., and H. Hayashi. 1993. Role of encystment and excystment of *Peridinium bipes* f. *occulatum* (Dinophyceae) in freshwater red tides in Lake Kizaki, Japan. *Jour. Phycol.*, 29(4):435–441.

Pérez-Martínez, C., and P. Sánchez-Castillo. 2001. Temporal occurrence of *Ceratium hirundinella* in Spanish reservoirs. *Hydrobiologia*, 452:101–107.

Pérez-Martínez, C., and P. Sánchez-Castillo. 2002. Winter dominance of *Ceratium hirundinella* in a southern north-temperate reservoir. *Jour. Plank. Res.*, 24(2):89–96.

Pfiester, L.A., R.A. Lynch, and J.J. Skvarla. 1980. Occurrence, growth, and SEM portrait of *Woloszynskia reticulata* Thompson (Dinophyceae). *Trans. Am. Micros. Soc.*, 99(2):213–217.

Pollingher, U. 1987. Freshwater ecosystems. In *The biology of dinoflagellates.* F.J.R. Taylor, ed. Oxford: Blackwell Scientific.

Pollingher, U., and B. Hickel. 1991. Dinoflagellate associations in a subtropical lake (Lake Kinneret, Israel). *Arch. Hydrobiol.*, 120:267–285.

Pollingher, U., H.R. Bürgi, and H. Ambühl. 1993. The cysts of *Ceratium hirundinella*: Their dynamics and role within a eutrophic (Lake Sempach, Switzerland). *Aquat. Sci.*, 55:10–18.

Popovský, J., and L.A. Pfiester. 1990. *Süßwasserflora von Mitteleuropa.* Band 6: Dinophyceae (Dinoflagellida). Stuttgart: Gustav Fischer Verlag.

Roset, J., A. Gibello, S. Aguayo, L. Dominguez, M. Álvarez, J.F. Fernández-Garayzabal, A. Zapata, and M.J. Muñoz. 2002. Mortality of rainbow trout [*Oncorynchus mykiss* (Walbaum)] associated with freshwater dinoflagellate bloom [*Peridinium polonicum* (Woloszynska)] in a fish farm. *Aquacult. Res.*, 33:141–145.

Sako, Y., Y. Ishida, H. Kadota, and Y. Hata. 1984. Sexual reproduction and cyst formation in the freshwater dinoflagellate *Peridinium cunningtonii. Bull. Jpn. Soc. Sci. Fish.*, 50:743–750.

Shei, P., T. Iwakuma, and K. Fujii. 1988. Feeding of *Chaoborus flavicans* larvae (Diptera: Chaoboridae) on *Ceratium hirundinella* and *Daphnia rosea* in a eutrophic pond. *Jpn. Jour. Limnol.*, 49:227–236.

Starmach, K. 1974. *Flora slodkowodna Polski 4: Cryptophyceae, Dinophyceae, Raphidiophyceae.* Warszwa, Kraków: PWN.

Stelzer, C.P. 1998. Feeding behavior of the rotifer *Ascomorpha ovalis*: functional response, handling time and exploitation of individual *Ceratium* cells. *Jour. Plank. Res.*, 20(6):1131–1144.

Stewart, A.J., and D.W. Blinn. 1976. Studies on Lake Powell, USA: Environmental factors influencing phytoplankton success in a high desert warm monomictic lake. *Arch. Hydrobiol.*, 78(2):139–164.

Stoneburner, D.L., and L.A. Smock. 1980. Plankton communities of an acidic, polymictic, brown-water lake. *Hydrobiologia*, 69:131–137.

Van Ginkel, C.E., B.C. Hohls, and E. Vermaak. 2001. A *Ceratium hirundinella* (O.F. Müller) bloom in Hartbeespoort Dam, South Africa. *Water SA*, 27(2):269–276.

Wu, J-T., and J-W. Chou. 1998. Dinoflagellate associations in Feitsui Reservoir, Taiwan. *Bot. Bull. Acad. Sinica*, 39:137–145.

Wu, J-T., L-L. Kuo-Huang, and J. Lee. 1998. Algacidal effect of *Peridinium bipes* on *Microcystis aeruginosa. Curr. Microbiol.*, 37:257–261.

Yan, N.D., and P. Stokes. 1978. Phytoplankton of an acidic lake, and its responses to experimental alterations of pH. *Environ. Conserv.*, 5(2):93–100.

Yoshikawa, T., K. Takishita, Y. Ishida, and A. Uchida. 1997. Molecular cloning and nucleotide sequence analysis of the gene coding for chloroplast-type ferredoxin from the dinoflagellates *Peridinium bipes* and *Alexandrium tamarense. Fish. Sci*, 63:692–700.

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Chapter 9

Cryptophyta

Paul Kugrens and Brec Clay

Cryptomonads, as the members of the division (phylum) Cryptophyta commonly are called, represent a well-circumscribed group of unicellular flagellates. The unique features include the cell covering, the flagellar structure, the surface architecture of cells, pigmentation, ejectisomes, chloroplast structure, and the presence of a reduced red algal nucleus called the nucleomorph. Most of these variations were discovered by using specialized electron microscopic techniques (Hill 1990; Hill and Wetherbee 1988, 1989; Kugrens and Lee 1987; Kugrens et al. 1986). The identification of genera and species is difficult with light microscopy; therefore, proper species identification continues to depend upon the use of electron microscopic and molecular analyses. Nevertheless, this chapter provides basic information that should allow identification of genera using a light microscope.

Except for *Goniomonas* and *Kathablepharis*, cryptomonads have one of the most complex cells known, having originated from a eukaryote host cell and a eukaryotic endosymbiont (Archibald 2007, Lane et al. 2007). An endosymbiont is a permanent structure in a cell that evolved from free living organisms after they were ingested but not digested. The pigmented cryptomonads and *Chilomonas* have four genomes: the host nuclear genome, the mitochondrial genome, the chloroplast genome, and the nucleomorph genome. These associations apparently originated from three distinct symbiotic events, two prokaryote–eukaryote events, and a eukaryote–eukaryote endosymbiotic association (Ludwig and Gibbs 1985, Gillott and Gibbs 1980, McFadden 1990, Lane et al. 2007).

Cryptomonads are important primary producers in freshwater and marine habitats (Gillott 1990, Klaveness 1988, Kugrens and Clay 2003), and they are cosmopolitan in their distribution. Most are found in phytoplankton samples, but their cells are extremely delicate and rupture when fixatives are added or when temperatures are elevated. As a consequence, their numbers generally appear low in preserved samples, and living samples must be used for counts and identification. They often are the dominants in phytoplankton populations in temperate lakes and reservoirs where they may dominate the sub-ice, early spring, and late fall populations.

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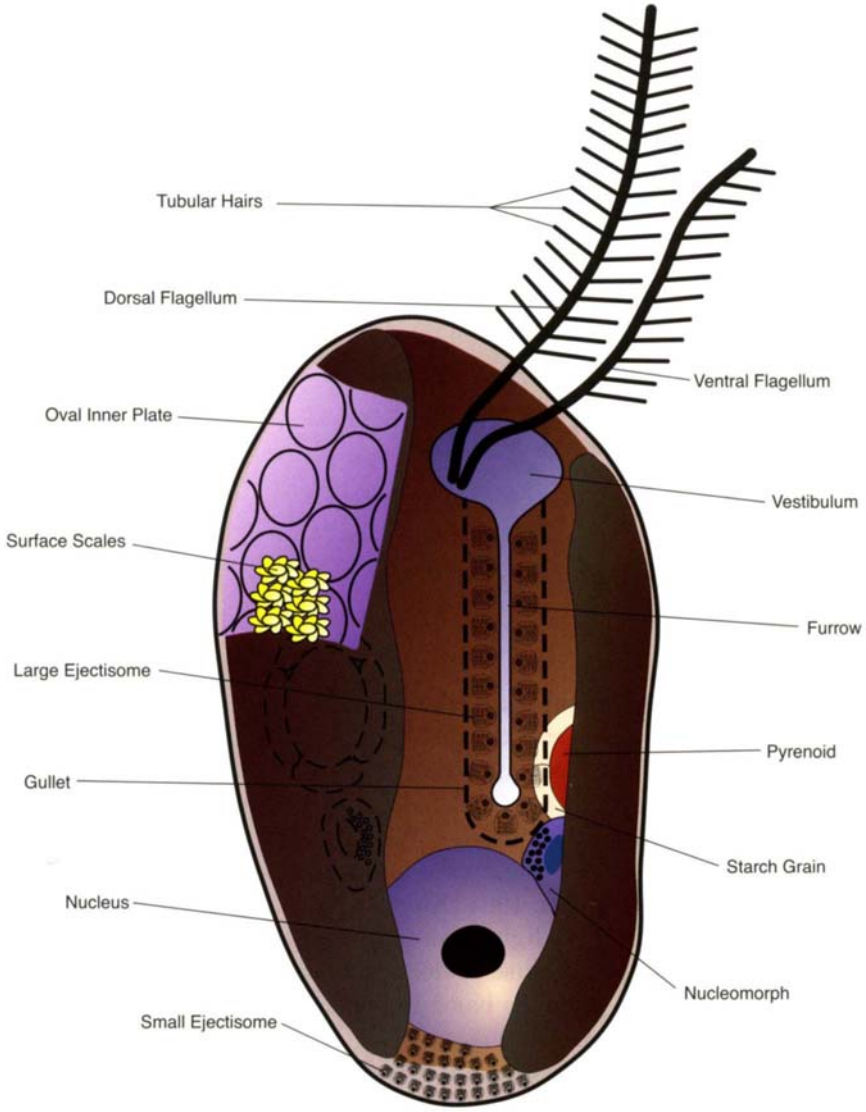

Figure 9-1 Diagram of a generalized cryptomonad cell showing the cellular details

BIOLOGY

Electron microscopic techniques need to be utilized whenever possible to determine accurately the many unique features of cryptomonads. By using the variations inherent in the furrow/gullet complex and the periplast, 18 genera of cryptomonads have been recognized. Most importantly, a correct interpretation of the actual structures must be made since the key characters, such as cell architecture and periplast, easily produce artifacts. Figure 9-1 illustrates the features found in cryptomonad cells, which are described in detail in this section.

Cell Shapes

Generally, cryptomonad cells have an asymmetric shape that is imparted by a furrow/gullet complex and the subapical, unilateral insertion of the flagella. Cell shapes may be oval, compressed, lunulate, caudate, acute, elongate, sigmoid, or contorted. All cells possess an anterior, outwardly facing depression called a vestibulum from which the

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flagella originate on the right side. In addition, there may be a gullet, some type of furrow, or a combination of a furrow-gullet.

Furrows and Gullets

A furrow is a ventral groove, of variable length, that begins in the vestibular region of the cell and extends posteriorly, terminating somewhere in the anterior half of the cell (Hill 1990; Hill and Wetherbee 1988, 1989; Klaveness 1985; Kugrens and Clay 2003; Kugrens et al. 1986; Munawar and Bistricki 1979). Several types of furrows have been described in cryptomonads (Hill and Wetherbee 1986, 1988, 1989; Kugrens et al. 1986; Munawar and Bistricki 1979), and these variations play a major role in cryptomonad systematics (Clay et al. 1999; Hill 1990, 1991a; Hill and Wetherbee 1986, 1988, 1989).

A tubular invagination called the gullet may extend posteriorly from the vestibulum or from the end of the furrow (Hill 1990, 1991a; Hill and Wetherbee 1986, 1988, 1989). Some genera only have a gullet or a furrow, while many genera have a combination of a furrow and gullet, and these may represent intermediate stages in the evolution of a gullet. Variations in these features are provided for each genus in the event that scanning electron microscopy is utilized.

Cell Covering

Except for *Kathablepharis*, the cell covering in cryptomonads is called a periplast. Periplasts consist of inner and surface components (Hill 1990; Hill and Wetherbee 1988, 1989; Kugrens and Lee 1987; Wetherbee et al. 1987; Wetherbee et al. 1986), and both can be variable in their composition. Whatever the structure of the inner periplast component (IPC), the component (IPC) is comprised of protein (Faust 1974) and may consist of multiple plates having various shapes or a single sheet (Hill 1990, 1991a; Hill and Wetherbee 1986, 1988, 1989; Kugrens and Clay 2003; Kugrens and Lee 1987). The surface periplast component may consist of plates, heptagonal scales, mucilage, or a combination of any of the preceding. The multiple plates of the IPC are connected to the cell membrane by intramembrane particles or proteins whereas the single sheet is not attached to the plasma membrane, although the ejectisome vesicle chambers are docked to the plasma membrane by IMPs (Brett and Wetherbee 1986; Grim and Staehelin 1984; Kugrens and Lee 1987; Kugrens et al. 1994; Wetherbee et al. 1986, 1987). The arrangement of these IMP domains conforms to the plate shapes (Brett and Wetherbee 1986; Kugrens and Lee 1987, 1991; Wetherbee et al. 1987). Variations in the shapes of these plates have been used in establishing genera. Multiple plated periplasts may be hexagonal, square, oval/round, rectangular, or irregular-shaped plates. In multiple-plated genera, their presence can be detected with the light microscope, if the plates are large enough, although their shapes cannot be distinguished. *Kathablepharis* only has an outer covering of scales (Lee and Kugrens 1991a, 1991b), and flattened vesicles of endoplasmic reticulum occurs just underneath the plasma membrane, rather than plates.

Flagella

With the exception of *Goniomonas*, cells have two subapically inserted, subequal flagella with bipartite tubular hairs on at least one of the flagella (Kugrens et al. 1987). The most common arrangement of hairs on the flagella is one in which the longer or dorsal flagellum bears two laterally opposed rows of tubular hairs and the shorter or ventral flagellum bears a single row of hairs. Flagella in *Goniomonas* are inserted on the dorsal side of the vestibulum and bear short spikes instead of hairs (Kugrens and Lee 1991).

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Cloroplasts and Pigmentation

Chloroplasts may be olive green, brownish, blue-green, or red depending on the pigments present. Pigments consist of chlorophylls *a* and c_2, alpha and beta carotene, alloxanthin, diadinoxanthin, and several forms of blue and red phycobiliproteins called Cr-phycocyanin and Cr-phycoerythrin to differentiate them from cyanobacterial and rhodoprotist phycobiliproteins. One or two chloroplasts occur in the cells of pigmented genera (Hill 1990). Either Cr-phycocyanin or Cr-phycoerythrin (Hill and Rowan 1989) is located inside the lumens of the photosynthetic lamellae (Gantt et al. 1971). Chloroplasts are surrounded by two additional membranes called the periplastidial envelope, periplastidial compartment, periplastidial complex, starch compartment, or chloroplast endoplasmic reticulum, which is continuous with the outer membrane of the nuclear envelope and surrounds the chloroplast, starch grains, and the nucleomorph (Gillott and Gibbs 1980). Starch granules are formed within the cytoplasm of the red algal endosymbiont called the periplastidial compartment, not in the chloroplast. The photosynthetic membranes in the chloroplasts (thylakoids) usually are arranged in pairs (Kugrens and Clay 2003) or sometimes in groups of three (Klaveness 1981, Hill 1991b). The plastid in *Chilomonas* lacks pigments and is called a leucoplast (Kugrens and Lee 1991). *Goniomonas* and *Kathablepharis* lack plastids and nucleomorphs; consequently, they also lack the periplastidial compartment.

Ejectisomes

Ejectisomes are extrusive organelles in all cryptomonads, although their substructure differs in *Kathablepharis.* Two sizes of ejectisomes are found in cryptomonad cells (Kugrens et al. 1994). Large ejectisomes are associated with the furrow/gullet, and small ejectisomes are found beneath the periplast in other regions of the cell. Ejectisomes consist of two different-sized, tightly coiled and tapered ribbons, which are joined, and both are surrounded by a membrane. Upon discharge, the ribbons roll up to form a tube. The function of ejectisomes remains unknown and their discharge is rapid. Although their function remains unknown, their discharge may be an evasive mechanism from predators or a stinging mechanism to repel predators. The large ejectisomes are easily visualized by light microscopy as groups in the anterior to midportions of the cell.

Nucleomorphs

Nucleomorphs are located in the periplastidial compartment (Gillott and Gibbs 1980, Kugrens and Clay 2003, Ludwig and Gibbs 1985). They represent a vestigial nucleus, derived from a red algal ancestor (Archibald 2007, Douglas et al. 1991). The nucleomorph is small, it is limited by a double membrane, and it contains three chromosomes (Archibald 2007, Lane et al. 2007). Its location within the cell may have systematic applications, specifically in *Rhodomonas* and *Storeatula,* where the nucleomorph is located in the pyrenoidal bridge (Hill and Wetherbee 1989).

Pyrenoids

Pyrenoids are concentrated regions for the enzyme Ribulose bisphphospate carboxylase oxidase (Rubisco) that functions in fixing carbon dioxide. These generally appear as protrusions from chloroplasts surrounded by starch granules. In *Rhodomonas* and *Storeatula,* a prominent pyrenoid connects both lobes of a chloroplast. Most cryptomonads have pyrenoids, but for the purposes of this chapter, species that lack pyrenoids are not included.

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Reproduction

Reproduction usually is asexual and occurs by mitotic divisions, although sexual cycles have been documented for *Proteomonas* (Hill and Wetherbee 1986) and *Chroomonas* (Kugrens and Lee 1988). Cysts may also be produced to withstand adverse conditions (Santore 1978), although cyst formation is rare. Some species are capable of forming nonmotile pseudocolonies. Whether flagella are present or not, these aggregations of cells form extensive mucilage that keeps the cells together. More recently, evidence has been provided by Hoef-Emden and Melkonian (2003) that indicates that *Cryptomonas* and *Campylomonas* are diplomorphs of the same genus. One phase would be haploid whereas the other phase would be diploid, indicating that this also would represent a sexual mode of reproduction. Such a life history was first demonstrated by Hill and Wetherbee (1986) for the marine genus *Proteomonas*.

Nutrition

Cryptomonads are photoautotrophic, heterotrophic, or mixotrophic. Photoautotrophs synthesize organic molecules from CO_2 by photosynthesis. Heterotrophic cryptopomonads require some organic materials for their carbon source. Heterotrophs may be osmotrophic or phagotrophic. Osmotrophs utilize dissolved organic matter whereas phagocytes ingest particulate matter, including other organisms. Both types are restricted to colorless cryptomonads and kathablepharids. For instance, *Chilomonas* is colorless; thus it is unable to manufacture its own basic organic molecules. It is strictly osmotrophic and obtains organics by incorporating dissolved organic molecules into its cell for metabolism. It does not ingest particulate materials, which is known as phagotrophy, the type of nutrition that this alga was assumed to have. In fact, the cell covering is a major obstacle to phagotrophy. *Goniomonas*, on the other hand, is phagotrophic and routinely ingests bacteria (Kugrens and Lee 1991), presumably through a specialized structure in the cell known as the infundibulum (Hill 1991a, Kugrens and Lee 1991).

Mixotrophy occurs when a photosynthetic organism can also ingest particulate matter, primarily other cells, including prokaryotes and eukaryotes (Boraas et al. 1988). Although rare in cryptomonads, it has been reported in a few cryptomonads (Kugrens and Lee 1991, Tranvik et al. 1989). Indirect evidence suggests that *Cryptomonas* ingests bacteria (Tranvik et al. 1989), but electron microscopic examinations were not conducted. However, electron microscopy determined that one species of *Chroomonas* is mixotrophic (Kugrens and Lee 1991).

ECOLOGY

The cryptomonads have a cosmopolitan distribution and generally are most prominent in oligotrophic (low-nutrient) waters, although they also may be common in mesotrophic and eutrophic (high-nutrient) waters. Cooler water temperatures appear to favor the growth of these organisms, but they can also thrive in tropical waters. Some of the same species occur in marine and freshwater habitats, indicating that the same species can tolerate a broad range of salinities. Furthermore, they are adapted to low light intensities and low temperatures; thus, they can grow profusely under these conditions (Morgan and Kalff 1979). Survival under these conditions may be due to lowered respiration and lowered cell divisions. There appears to be a change to storing large amounts of starch, thereby increasing cell size (Morgan and Kalff 1975).

Cryptomonads display a wide range of pH tolerance, but generally the range of pH tolerance is narrow, and species appear to be restricted to a narrow pH range depending on the habitat in which they grow (Pringsheim 1968, Klaveness 1988). Our

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observations on cryptomonads in Colorado and Wyoming indicate that cryptomonads grow best in waters where pH values are above 7.5 (personal observations).

Cryptomonads are preferential food organisms for a variety of invertebrates (Guillard 1975, Klaveness 1984, Stemberger and Gilbert 1985, Sarnelle 1993, Li et al. 1996). In addition, they are preferentially ingested by colorless dinoflagellates, ciliates, and by *Kathablepharis* spp. (Stemberger and Gilbert 1985; Clay and Kugrens 1999a, 1999c; Lewitus et al. 1999). *Daphnia hyalina* and *Diaptomus gracilis* prefer *Rhodomonas* sp. when available (Ferguson et al. 1982). Furthermore, rotifers appear to select cryptomonads (Stemberger and Gilbert 1985), and the presence of cryptomonads enhances the reproduction of planktonic rotifers (Edmondson 1965). In addition, Pejler (1977) observed that various rotifers prefer *Rhodomonas* to *Chrysochromulina*. Given that many zooplankton selectively choose cryptomonads as prey, grazing probably plays a significant role in regulating planktonic cryptomonad population dynamics (Sarnelle 1993).

Kathablepharids are voracious predators, attacking their prey in groups ranging up to several hundred cells per group. They feed on both bacteria and various eukaryotes, but each freshwater species apparently prefers a specific food organism (Lee and Kugrens 1991a, 1991b). For instance, *Kathablepharis ovalis* feeds on the prymnesiophyte *Chrysochromulina parva,* whereas *K. phoenikoston* prefers the cryptomonad *Chroomonas* (Clay and Kugrens 1999c).

SIGNIFICANCE

Source Water

They often are the dominant phytoplankton in temperate lakes and reservoirs, where they may dominate the sub-ice, early spring, and late fall populations. Therefore, these algae would be most common in source water and could form blooms. However, they may also form nuisance blooms and can cause fish kills by depleting oxygen when they occur in large numbers.

Treatment Plants

Unless large blooms of mucilage-producing species occur in source waters, there should be minimal problems with these algae in water treatment plants. They are delicate and would rupture in any filtration or water treatment process.

Water Supply

As noted earlier, cryptomonads do not pose a health threat in water supplies and have not been reported to cause taste and odor problems. Since they are so delicate and rupture, they would not be detected even if they did cause taste and odor problems in the water.

Toxin Production

Generally, cryptomonads are not known as a group that produces toxins. A suspected cryptomonad, *Cyanomonas*, was implicated in massive catfish deaths in Texas ponds (Pfiester and Holt 1978). Neither the organism nor the toxin was isolated. The identity of *Cyanomonas* remains in doubt, because the quality of the light micrographs of the causal organism were not sufficient in detail to determine whether it indeed was *Cyanomonas* in particular or a member of the cryptomonads in general.

Halevy et al. (1971) reported that an ichthyotoxin similar to one produced by the chrysophyte *Ochromonas* was isolated from the colorless cryptomonad *Chilomonas*

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paramecium, but the cells produced only small amounts and would not be a factor in fish kills. Since it is not photosynthetic, it would not bloom in low-nutrient waters where fish would be affected. Cells produce this toxin in very small amounts, and there have not been any reports of fish kills involving this species.

An unidentified species of *Rhodomonas* also has been implicated in exotoxin production (Stemberger and Gilbert 1985), although it was not demonstrated that the inhibition of rotifer growth was due to *Rhodomonas*. The causative agents in the cultures may have been due to bacteria.

In May 2002, a massive fish kill in Prewitt Reservoir in Colorado was attributed to the golden alga *Prymnesium parvum* Carter (*Denver Post*, June 5, 2002); however, water samples a week later did not contain any cells of *P. parvum*. Instead there was a massive bloom of *Campylomonas reflexa* and *Plagioselmis nanoplanctica*. There were more than 4 million cells/mL of *C. reflexa* and more than 2.5 million cells/mL of *P. nanoplanctica*, which could have caused the deaths by removing oxygen from the water during the night, causing anoxia.

KEY FOR IDENTIFICATION OF CRYPTOMONAD GENERA

Systematics of Cryptomonads

The systematics and diversity of freshwater cryptomonads remain uncertain, but both have been enhanced through the use of electron microscopic and molecular studies. A framework of useful characters and genes, as well as the proper techniques (quick-freezing and freeze-drying) need to be utilized to delineate accurately the genera of cryptomonads. Due to their unicellularity and small size, current and future classification schemes for cryptomonads must continue to rely on ultrastructural and molecular features. Currently, 18–20 genera of cryptomonads are recognized, with 11 genera found in freshwater. For the purposes of this chapter, the key is an artificial one that is intended to assist light microscopists in identifying these organisms to genus.

It must be noted that *Kathablepharis* is a distant relative of cryptomonads, because this group possesses unique characteristics that are distinct from those of cryptomonads. Okamoto and Inouye (2005) placed *Kathablepharis* spp. and *Leucocryptos* spp. in the newly proposed division Kathablepharidophyta Okamoto and Inouye. Since there is no other chapter devoted to these unique organisms, they are included with the cryptomonads. Ejectisomes are the only common feature between the two, and historically they have been included with cryptomonads. Again, electron microscopy and molecular analyses determined their proper phylogenetic placement.

Cryptomonas and *Campylomonas* are the most common freshwater genera and grow exclusively in freshwater. *Komma caudata* is the only blue-green colored species and also is found exclusively in freshwater. Any other blue-green colored cryptomonads are placed in the genus *Chroomonas*. However, it is more difficult to distinguish *Rhodomonas* from *Storeatula* with the light microscope. Both are reed colored, and by using oil immersion and differential interference microscopy (DIC), multiple plates can be distinguished for *Rhodomonas*. The remaining cell structure would be the same. The colorless genera are distinctive and should be easily identified with light microscopy. Detailed descriptions are provided for each genus. A key reference for identifying freshwater cryptomonad species is Kugrens and Clay (2003).

1a. Cells colorless, 2

1b. Cells pigmented, 4

2a. Cells with numerous starch grains, *Chilomonas*

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2b. Cells lacking starch grains, 3

3a. Cells with ejectisomes in a ring around anterior of cell, *Goniomonas*

3b. Ejectisomes in two discreet rows on ventral side of cell, *Kathablepharis*

4a. Cells blue-green in color, due to presence of phycocyanin, 5

4b. Cells olive, brown, or red in color due to presence of phycoerythrin, 7

5a. Flagella inserted approximately one-third of the cell length behind anterior, *Hemiselmis*

5b. Subapically inserted flagella, near anterior end, 6

6a. Cells comma-shaped with an acute posterior, *Komma*

6b. Cells oval, elongate, not comma-shaped, *Chroomonas*

7a. Cells not comma-shaped, 8

7b. Cells comma-shaped, *Plagioselmis*

8a. Cells with prominent central pyrenoid connecting both chloroplast lobes, 9

8b. Cells with two or more pyrenoids, 10

9a. Plates absent, *Storeatula*

9b. Multiple plates evident, *Rhodomonas*

10a. Cells oval, ovoid, or obovoid, flattened dorsiventrally, *Cryptomonas*

10b. Cells contorted/twisted, *Campylomonas*

DESCRIPTIONS OF GENERA

***Chilomonas* Ehrenberg** (Figure 9-2). Cells have a rostrate anterior, are colorless, and are not phagocytic. Furrow/gullet complex consists of a vestibulum, short furrow, and long tubular gullet; a vestibular ligule covers the area of contractile vacuole discharge. Both flagella have a unilateral row of tubular hairs. The periplast has an inner single sheet with numerous ejectisome pores and a surface periplast component consisting primarily of fibrils. Two small, colorless leucoplasts are present within the periplastidial compartment, containing numerous large starch grains and two nucleomorphs. The genus is strictly freshwater. Cells measure 15–40 μm in length. For additional information, refer to Anderson (1962), Grim and Staehelin (1984), Kugrens and Clay (2003), Kugrens and Lee (1987, 1991), and Kugrens et al. (1986).

***Goniomonas* Stein** (Syn. *Cyathomonas*) (Figure 9-3). Cells are laterally compressed, colorless, sometimes phagocytic, and containing bacteria. Flagella are inserted on the dorsal side of the cell, one flagellum with recurved spines, the other flagellum with fine fibrillar hairs. Large ejectisomes form a distinctive band around the anterior portion of the cell. The periplast is comprised of inner and outer rectangular plates that are not offset. These plates can be resolved with DIC microscopy. Chloroplasts or leucoplasts are lacking. This genus is freshwater and marine. Cells measure 5–15 μm in length. For additional information, refer to Hill (1991a), Kugrens and Lee (1991), Mignot (1965), and Kugrens and Clay (2003).

***Kathablepharis* Skuja** (Figure 9-4). Cells are colorless and phagocytic, and the flagella are inserted subapically. Cells have a complex ingestion or feeding apparatus that resembles an apical complex (Lee et al. 1991). Several species are common flagellates in freshwater habitats, especially in the Rocky Mountain region. *K. ovalis*, the

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A B

Figure 9-2 *Chilomonas*: (A) scanning electron micrograph, (B) diagram

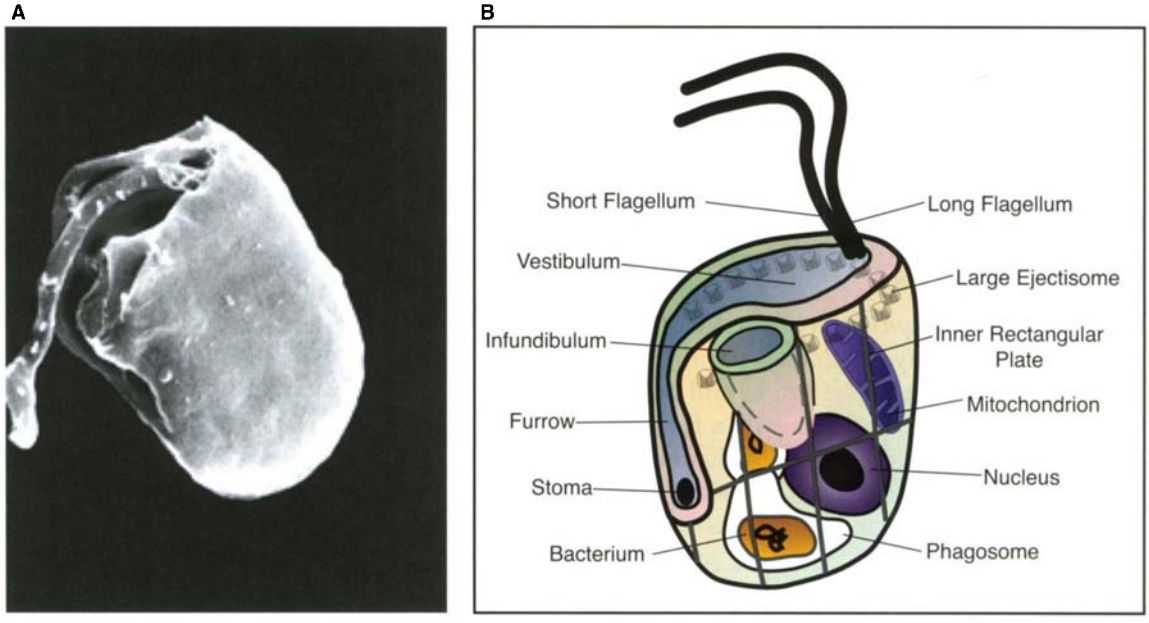

Figure 9-3 *Goniomonas*: (A) scanning electron micrograph, (B) diagram

most common species, preferentially feeds on the prymnesiophyte, *C. parva*, making it appear as though cells contain golden-colored chloroplasts, which actually are prey cells or chloroplasts of the prey. The cells have a large central nucleus, with the food vacuole located behind the nucleus. One to several large food vacuoles occupy the posterior portion of the cell. Large ejectisomes occur in two rows posterior to, and just

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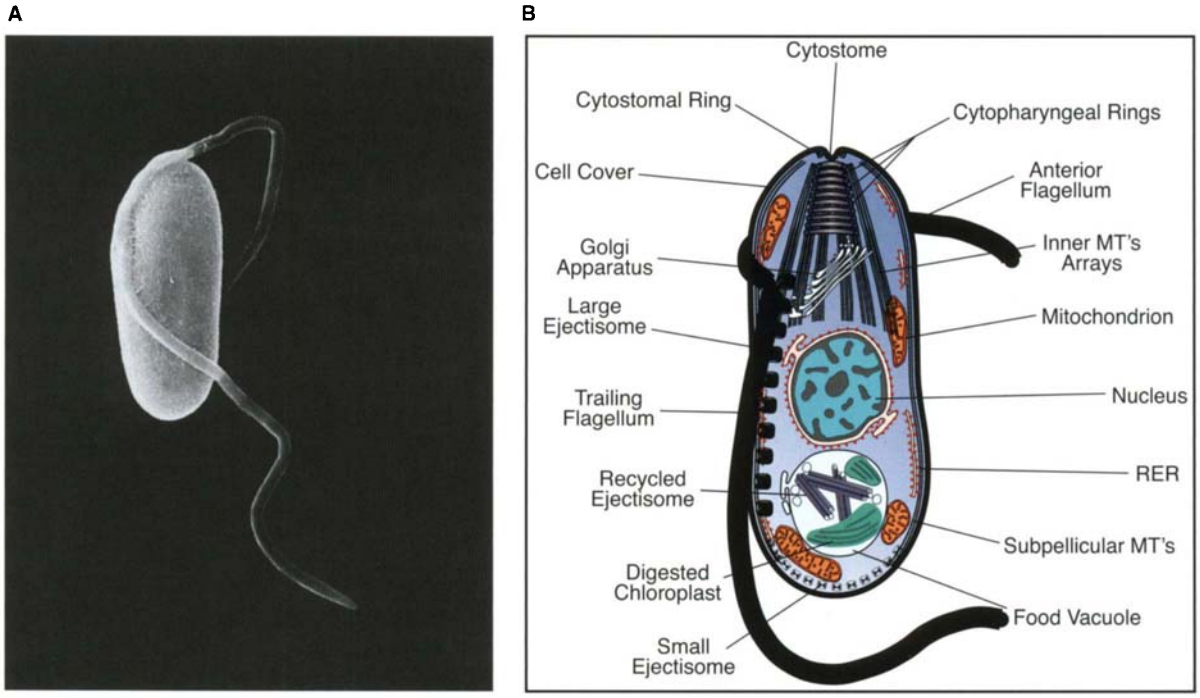

Figure 9-4 *Kathablepharis:* (A) scanning electron micrograph, (B) diagram

to the right of, the flagella, while smaller ejectisomes occur under the plasma membrane in the posterior and medial portion of the cell. This organism, however, is easily overlooked because it is small, colorless, and has no striking features when examined with the light microscope. In addition to dispersed cells, swarms of *Kathablepharis* are common, consisting of aggregations of 20–100 cells that attack a specific prey cell. *Kathablepharis* is freshwater and marine, and cells measure 5–15 µm in length. For further information, refer to Kugrens and Clay (2003), Lee and Kugrens (1991a), Clay and Kugrens (1999a, 1999b), and Okamoto and Inouye (2005). In fact, based on the cytological descriptions and molecular data, Okamoto and Inouye (2005) proposed a new phylum for this group of protists to which *Kathablepharis* belongs.

***Hemiselmis* Parke** (Figure 9-5). Cells are rounded anteriorly and posteriorly and may be dorsoventrally flattened, appearing somewhat bean-shaped in lateral view. The vestibulum and gullet are located approximately one-third of the cell length from the anterior, and a furrow is absent. Inner and surface periplast components are comprised of large hexagonal plates. Cells contain a single dorsal, blue-green chloroplast with a centrally situated, large pyrenoid and a nucleomorph located anterior to the pyrenoid. Chloroplasts contain Cr-phycoerythrin 555, imparting a blue-green color to the cells. However, some red-colored species occur only in marine habitats. Only *H. amylosa* Clay and Kugrens (1999b) has been described from freshwater. For additional information, refer to Kugrens and Lee (1987), Pennick (1981, 1982), Wetherbee et al. (1986), Hill (1990), Kugrens and Clay (2003), and Kugrens et al. (1986).

***Komma* Hill** (Figure 9-6). Cells are comma-shaped or acuminate with a rounded anterior end, tapering to a pointed or acutely rounded posterior. A furrow is lacking but a tubular gullet extends posteriorly from the base of the vestibulum. The periplast consists of relatively small internal and surface hexagonal plates. Each cell has a single blue-green chloroplast on the dorsal side of the cell with a central pyrenoid projecting from the chloroplast. The chloroplast contains C-phycocyanin 645. The genus is

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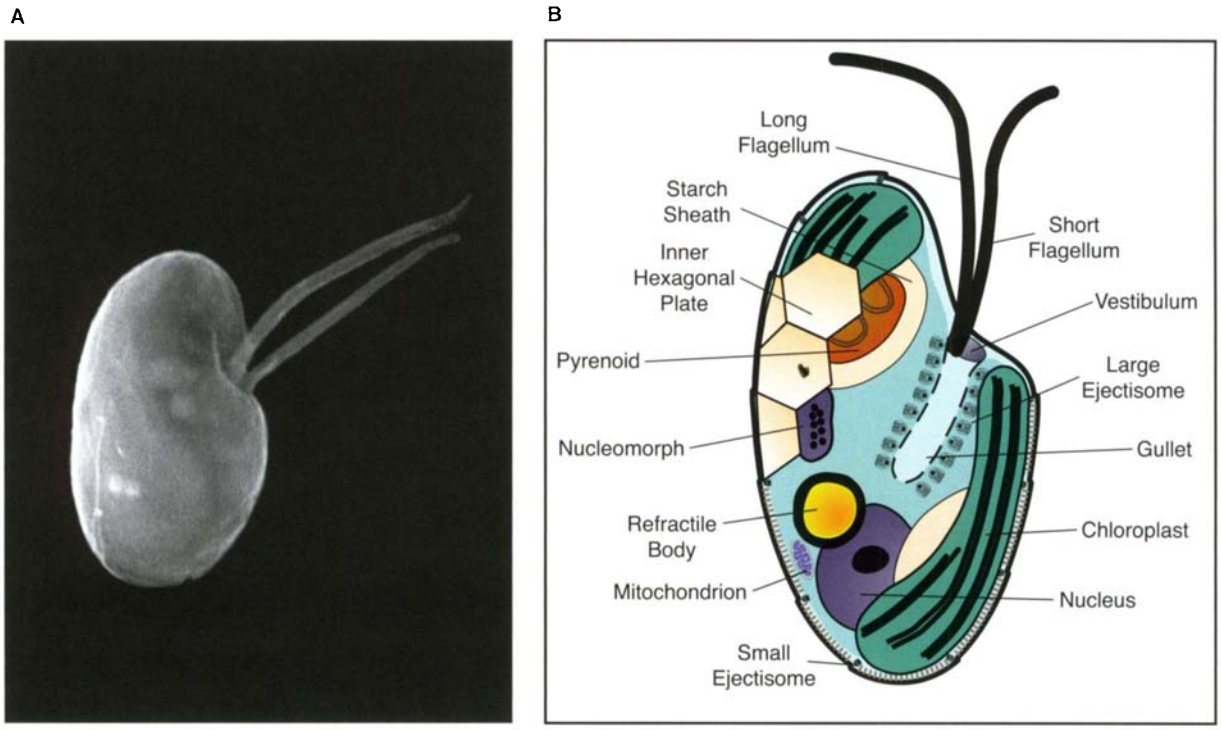

Figure 9-5 *Hemiselmis:* (A) scanning electron micrograph, (B) diagram

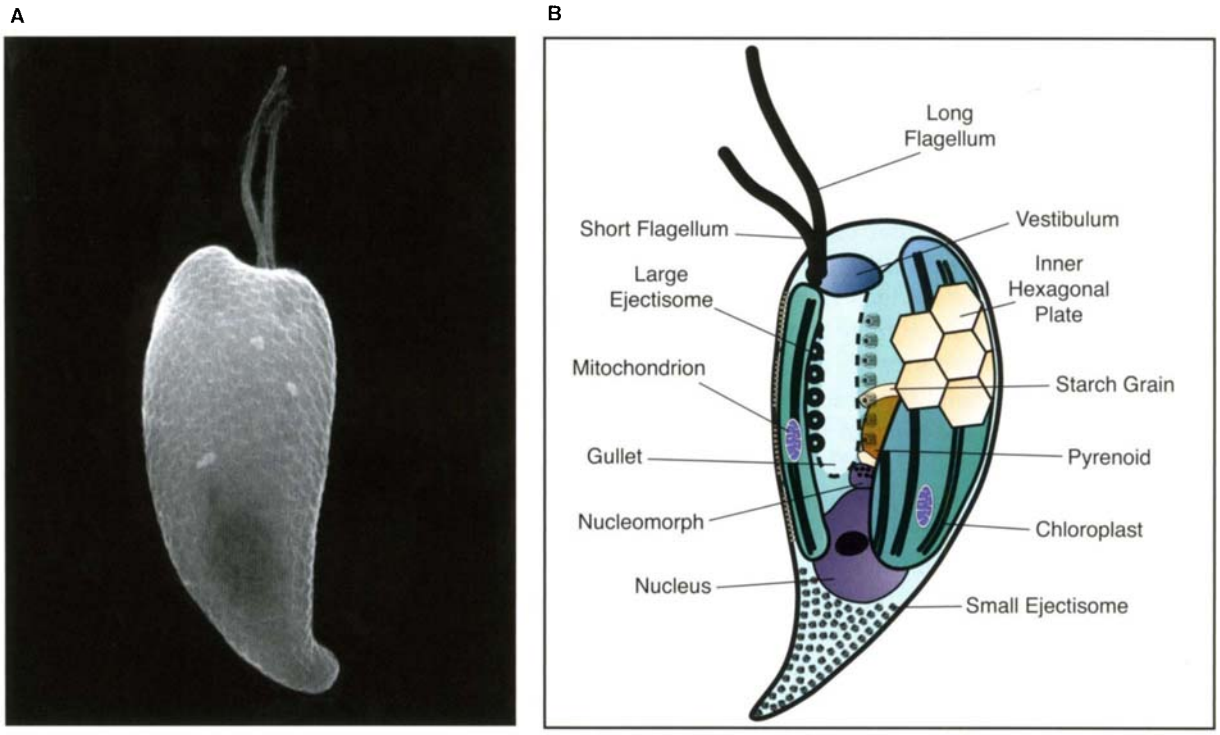

Figure 9-6 *Komma:* (A) scanning electron micrograph, (B) diagram

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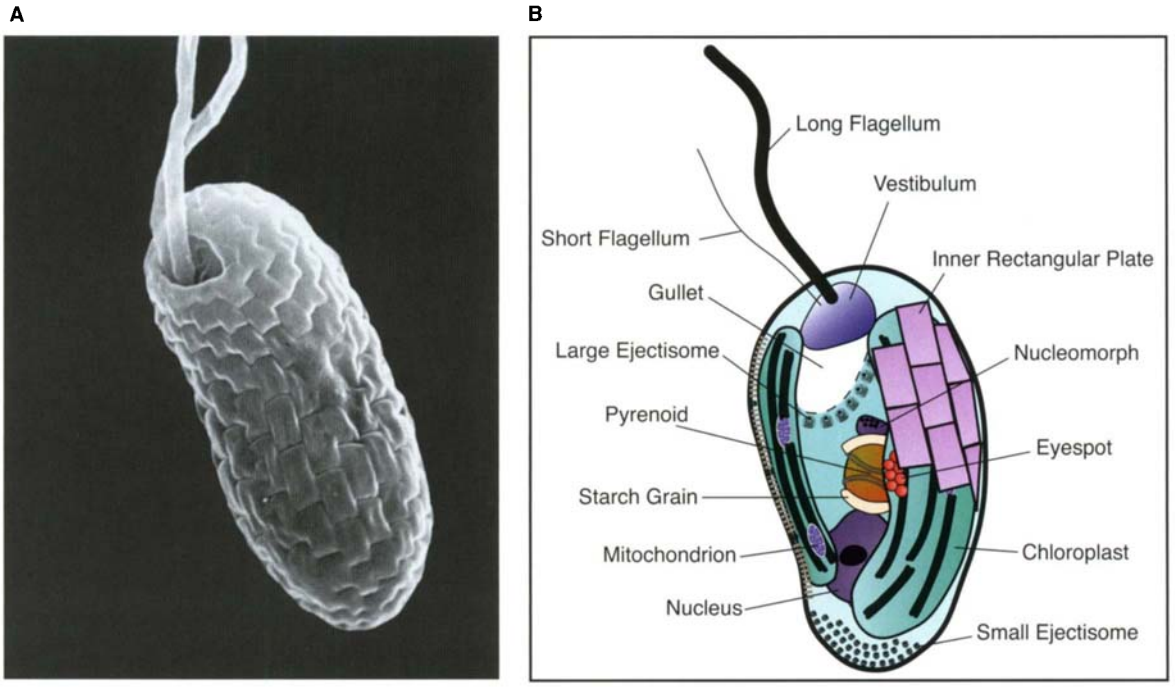

Figure 9-7 *Chroomonas:* (A) scanning electron micrograph, (B) diagram

monospecific (*K. caudata* Hill), and it is strictly freshwater. For further information, refer to Hill (1990), Kugrens and Clay (2003), Kugrens and Lee (1987), and Kugrens et al. (1986).

***Chroomonas* Hansgirg** (Figure 9-7). Cells are subobovate or barrel shaped and frequently form pseudocolonies by extensive mucilage production, with the mucilage encasing the cells and causing them to attach to various substrates. *Chroomonas* spp. in streams and rivers commonly occur in attached colonies. Cells lack a furrow but a tubular gullet extends posteriorly from the vestibulum. Inner and outer components of the periplast consist of offset rectangular plates (Hill 1990), with the anterior of the plate edges raised due to rows of intramembrane particles in the cell membrane adhering tightly to the plates in the posterior end of each plate. Cells have a single chloroplast with a prominent pyrenoid. The nucleomorph usually is located near the pyrenoid. Contractile vacuoles in freshwater species are situated in the anterior. Chloroplasts contain either Cr-phycocyanin 645 or 630, imparting a blue-green color to the cells. A reddish eyespot or stigma may be present in chloroplasts of some species, such as the freshwater *C. coerulea* (Geitler) Skuja. This genus is either freshwater or marine. For further information on *Chroomonas*, refer to Antia et al. (1973), Dodge (1969), Gantt et al. (1971), Hill (1990), Kugrens and Clay (2003), Kugrens and Lee (1987), Kugrens et al. (1986), Meyer and Pienaar (1984), and Novarino (1993).

***Plagioselmis* Butcher** (Figure 9-8). This genus originally was described from the marine environment (Butcher 1967) until Novarino et al. (1994) transferred the freshwater *Rhodomonas minuta* into the genus *Plagioselmis*, as *P. nanoplanctica*. Cells are pink, red, or brown in coloration due to the presence of phycoerythrin, and they are comma-shaped with an acute tail that lacks plates. Cells have a prominent ventral furrow. The periplast consists of an internal component (IPC) of hexagonal plates. *P. nanoplanctica* (Skuja) Novarino is the only recognized freshwater species. Cells range in size from 12 to 18 μm in length and from 5 to 8 μm in diameter at the widest portion of the cell.

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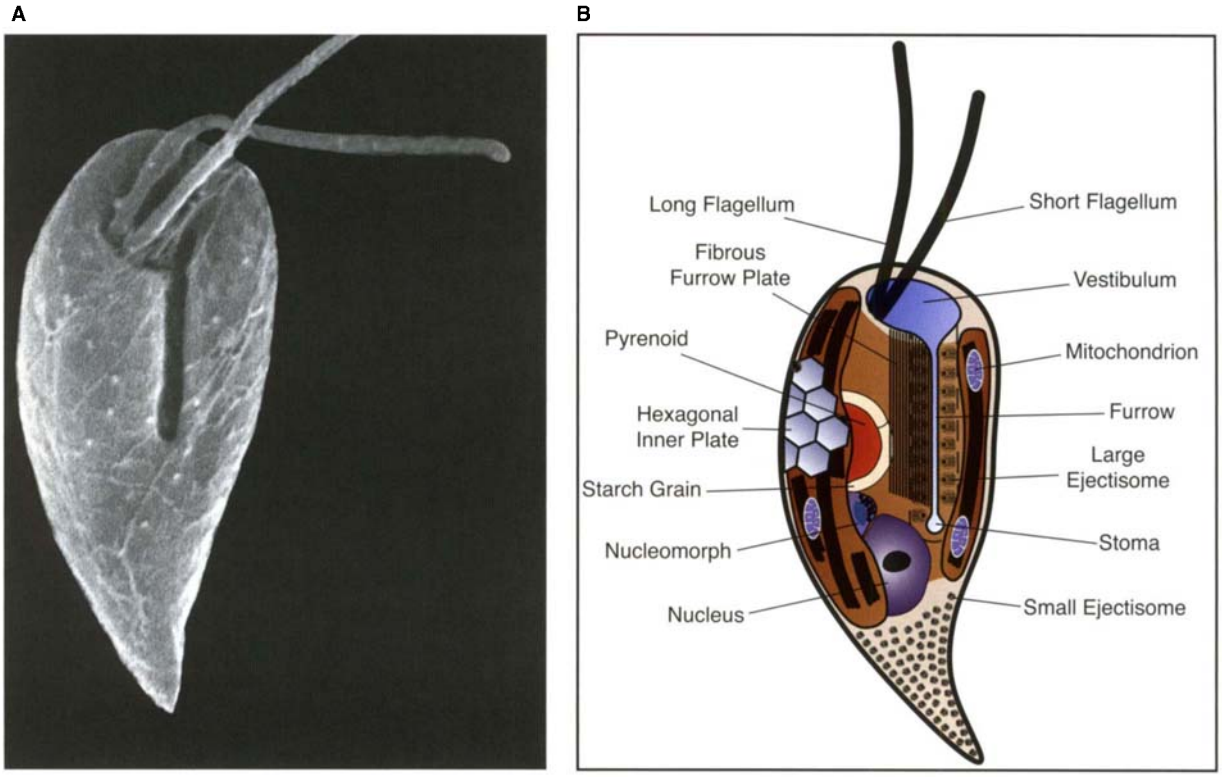

Figure 9-8 *Plagioselmis:* (A) scanning electron micrograph, (B) diagram

***Storeatula* Hill** (Figure 9-9). Cells are ellipsoid with a slightly pointed anterior and are red to pink in coloration. A furrow is lacking, and the gullet extends to approximately the middle of the cell and is lined with several rows of ejectisomes. The periplast has an inner sheet and an outer component of a thick mat of coarse fibrils. Cells contain a single lobed chloroplast, a pyrenoid that connects both lobes of the chloroplast, and a nucleomorph located in an anterior groove or depression in the pyrenoid. With the light microscope it is difficult to distinguish *Storeatula* from *Rhodomonas*, unless DIC microscopy is utilized, which can resolve multiple plates in *Rhodomonas*. Chloroplasts contain the biliprotein Cr-phycoerythrin 545. One species, *S. rhinosa*, has been described from freshwater (Kugrens et al. 1999). For additional information, refer to Hill (1991b), Kugrens and Clay (2003), and Kugrens et al. (1999).

***Rhodomonas* Karsten** (= *Pyrenomonas* Santore) (Figure 9-10). Cells are oval, usually red- to pink-colored with a furrow of variable length and a short, tubular gullet. The periplast consists of inner, more or less square plates, which taper toward the anterior. Cells have a single lobed chloroplast with a pyrenoid situated between the two lobes of the chloroplast. A nucleomorph is located in an invagination of the pyrenoid. Chloroplasts contain phycoerythrin 545. The genus is found in marine and freshwater habitats. Only *R. ovalis* Skuja has been verified as a *Rhodomonas* from freshwater (Kugrens et al. 1999). For further information, refer to Erata and Chihara (1989), Hill and Wetherbee (1989), Novarino (1993), Santore (1984), and Kugrens and Clay (2003).

***Cryptomonas* Ehrenberg** (Figure 9-11). Cells are oval to obovoid and brownish in coloration, often forming mucilaginous palmelloid pseudocolonies, with cells surrounded by extensive mucilage. This genus has the most complex type of vestibular furrow/gullet complex, with the furrow consisting of furrow ridges, furrow folds, and

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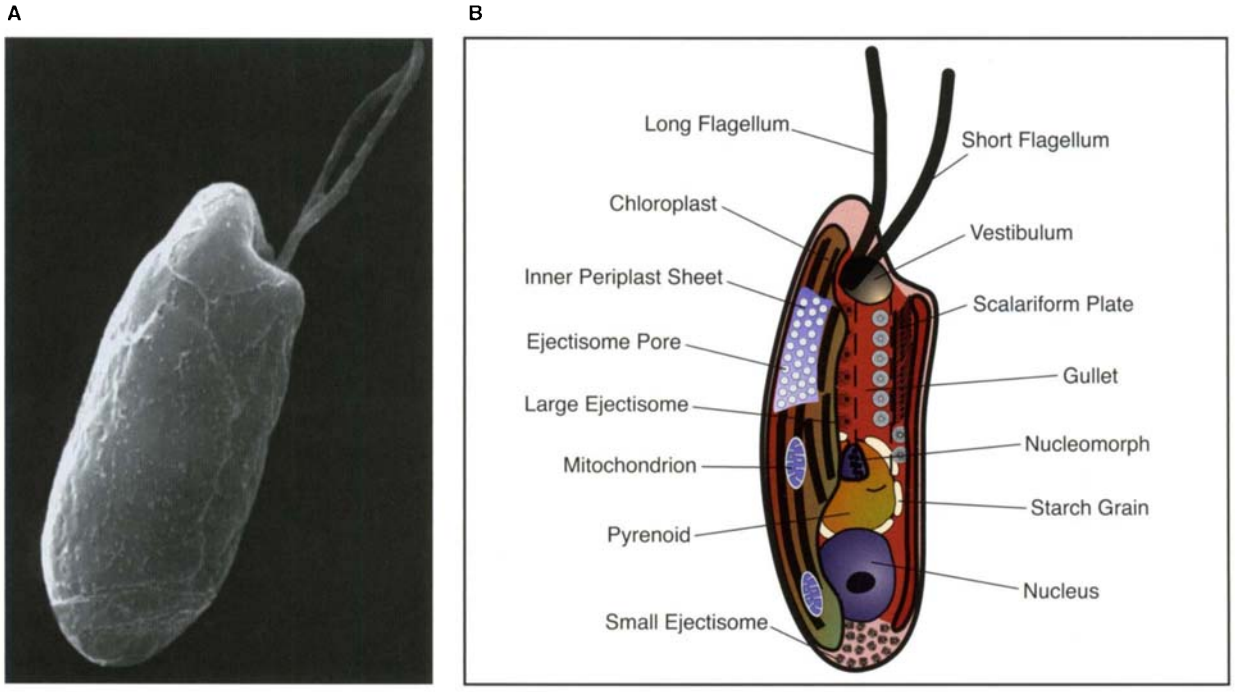

Figure 9-9 *Storeatula:* (A) scanning electron micrograph, (B) diagram

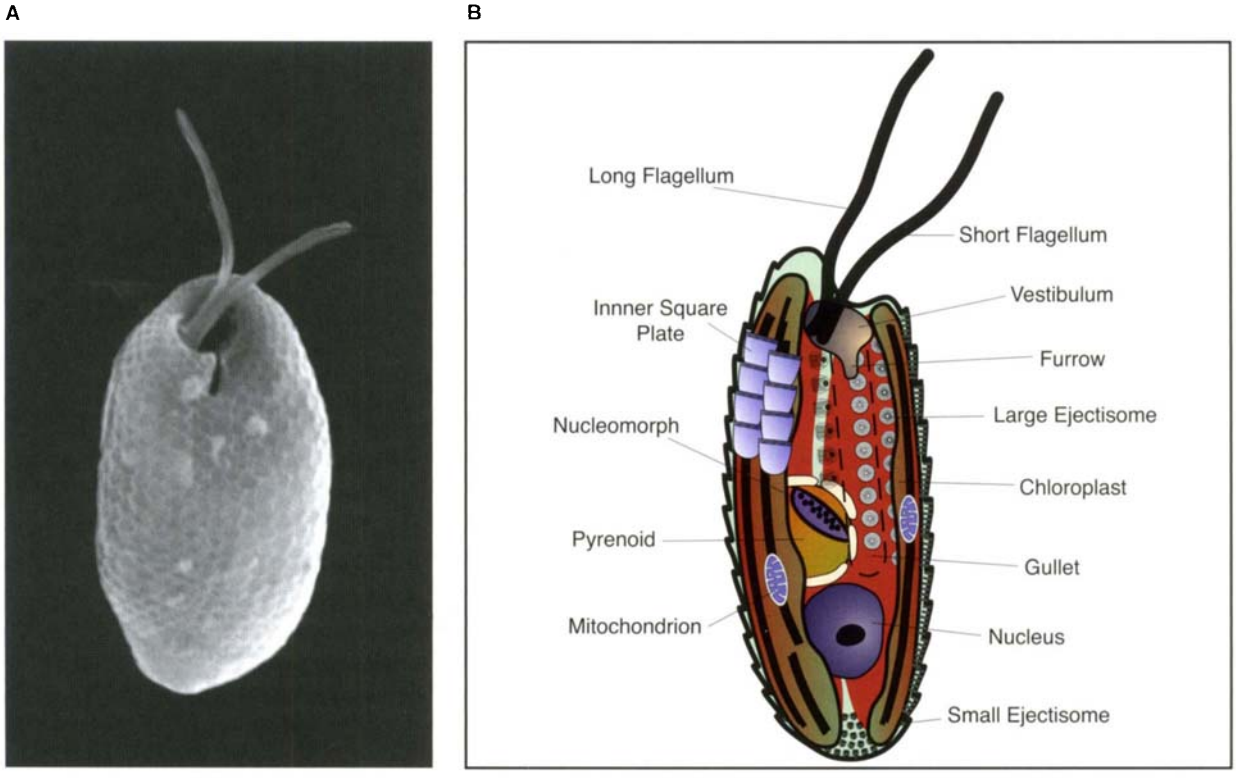

Figure 9-10 *Rhodomonas:* (A) scanning electron micrograph, (B) diagram

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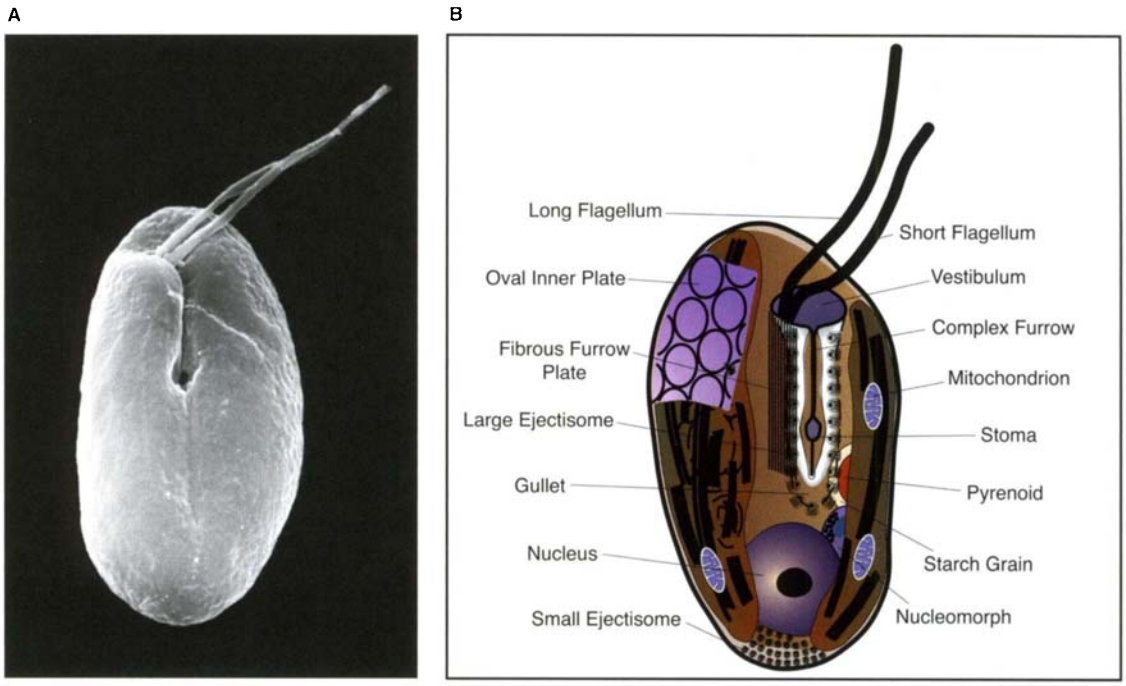

Figure 9-11 *Cryptomonas:* (A) scanning electron micrograph, (B) diagram

a persistent oval opening called the stoma, which is located near the posterior of the furrow (Kugrens et al. 1986). The periplast consists of an inner component of round or oval-shaped to hexagonal plates and a surface component of a thin layer of fibrils. Cells have two chloroplasts, each with one to two pyrenoids, depending on the species. Two nucleomorphs occur in each cell, and these are not embedded within the pyrenoids. Chloroplasts possess Cr-phycoerythrin 566. This genus has the most species, and it is strictly freshwater. The most common species is *C. ovata*. For additional information, refer to Brett and Wetherbee (1986), Hill (1991b), Kugrens and Lee (1987), Kugrens et al. (1986), Munawar and Bistricki (1979), Roberts (1984), and Santore (1977, 1984).

***Campylomonas* Hill** (Figure 9-12). Cells have a characteristic contorted shape, with some species having a recurved posterior that may be acuminate or flat, imparting a sigmoid shape to the cells in lateral view. The periplast consists of an inner sheet, but a surface periplast component appears to be lacking. Cells contain two chloroplasts, each with its own pyrenoid and nucleomorph located near each pyrenoid. *C. rostratiformis* is the largest species among the cryptomonads and possesses several pyrenoids per chloroplast. Chloroplasts possess Cr-phycoerythrin 566, and cells are brownish in coloration. This genus is strictly freshwater and may be an alternate form in the reproductive cycle of some *Cryptomonas* species (Hoef-Emden and Melkonian 2003). For more detailed information, refer to Hill (1991b), Klaveness (1985), Kugrens and Lee (1987), Kugrens et al. (1986), Munawar and Bistricki (1979), Hoef-Emden and Melkonian (2003), and Kugrens and Clay (2003).

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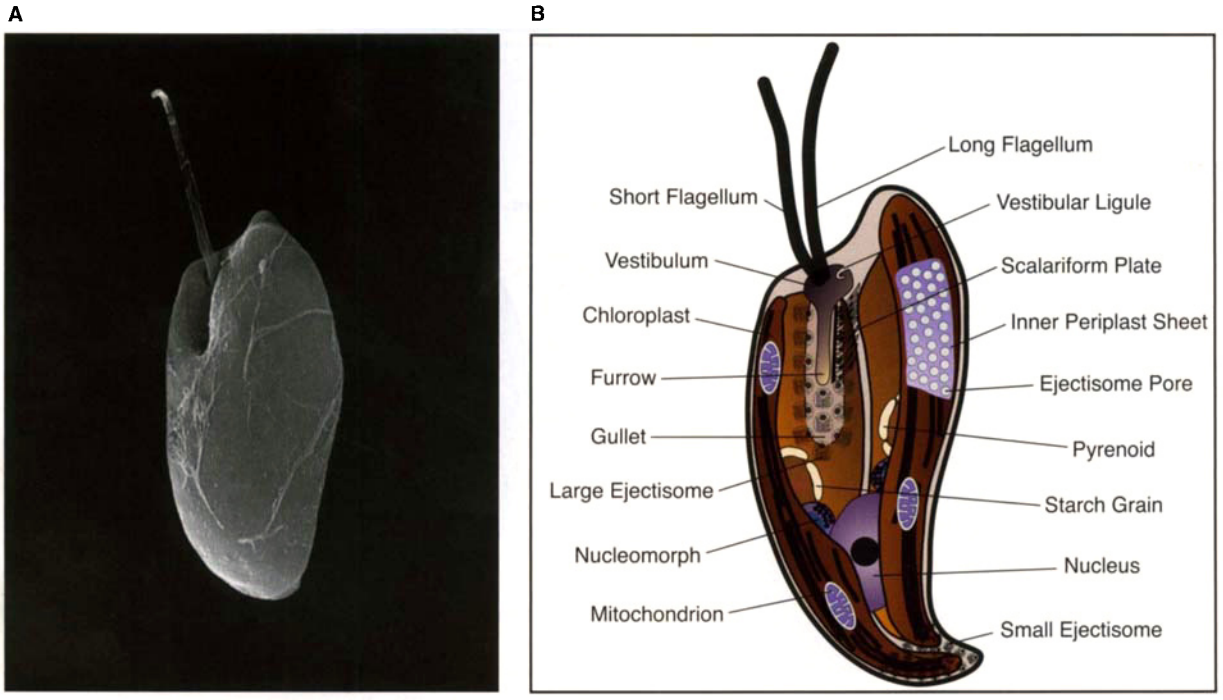

Figure 9-12 *Campylomonas:* (A) scanning electron micrograph, (B) diagram

REFERENCES

Anderson, E. 1962. A cytological study of *Chilomonas paramecium* with particular reference to the so-called trichocysts. *Jour. Eukaryot. Microbiol.*, 9:380–395.

Antia, N.J., J.P. Kalley, J. McDonald, and Z. Bisalputra. 1973. Ultrastructure of the marine cryptomonad *Chroomonas salina* cultured under conditions of photoautotrophy and glycerolheterotrophy. *Jour. Protozool.* 20: 377–385.

Archibald, J.M. 2007. Nucleomorph genomes: Structure, function, origin and evolution. *BioEssays*, 29:392–402.

Boraas, M.E., K.W. Estep, P.W. Johnson, and J.M. Sieburth. 1988. Phagotrophic phototrophs: The ecological significance of mixotrophy. *Jour. Eukaryot. Microbiol.*, 35:249–252.

Brett, S.J., and R. Wetherbee. 1986. A comparative study of periplast structure in *Cryptomonas cryophila* and *C. ovata* (Cryptophyceae). *Protoplasma*, 131:23–31.

Butcher, R. 1967. *An introductory account of the smaller algae of British coastal waters.* Part IV: Cryptophyceae. Fishery Investigations Series IV. London: Her Majesty's Stationery Office.

Clay, B.L., and P. Kugrens. 1999a. Systematics of the enigmatic kathablepharids, including EM characterization of the type species, *Kathablepharis phoenikoston*, and new observations on *K. remigera* comb. nov. *Protist*, 150:43–59.

Clay, B.L., and P. Kugrens. 1999b. Characterization of *Hemiselmis amylosa* sp. nov. and phylogenetic placement of the blue-green cryptomonads *H. amylosa* and *Falcomonas daucoides*. *Protist*, 150:297–310.

Clay, B.L., and P. Kugrens. 1999c. Description and ultrastructure of *Kathablepharis tenuis* sp. nov. and *K. obesa* sp. nov.—Two new freshwater kathablepharids (Kathablepharididae) from Colorado and Wyoming. *Eur. Jour. Protistol.*, 35:435–447.

Clay, B.L., P. Kugrens, and R.E. Lee. 1999. A revised classification of Cryptophyta. *Bot. Jour. Linn. Soc.*, 131:131–151.

Dodge, J.D. 1969. A review of the fine structure of algal eyespots. *Brit. Phycol. Jour.*, 4:199–210.

Douglas, S.E., C.A. Murphy, D.F. Spencer, and M.W. Gray. 1991. Cryptomonad algae are evolutionary chimaeras of two phylogenetically distinct unicellular eukaryotes. *Nature*, 350:148–151.

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Edmondson, W.T. 1965. Reproductive rate of planktonic rotifers as related to food and temperature in nature. *Ecol. Monogr.*, 35:61–111.

Erata, M. and M. Chihara. 1989. Re-examination of *Pyrenomonas* and *Rhodomonas* (Class Cryptophyceae) through ultrastructural survey of red pigmented cryptomonads. *Bot. Mag. Tokyo* 102:429–442.

Faust, M.A. 1974. Structure of the periplast of *Cryptomonas ovata* var. palustris. *Jour. Phycol.*, 10:121–124.

Ferguson, A.J.D., J.M. Thompson, and C.S. Reynolds. 1982. Structure and dynamics of zooplankton communities maintained in closed systems, with special reference to the algal food supply. *Jour. Plank. Res.*, 4:523–543.

Gantt, E., M.R. Edwards, and L. Provasoli. 1971. Chloroplast structure of the Cryptophyceae, evidence for phycobiliproteins within intrathylakoidal spaces. *Jour. Cell Biol.*, 48:280–290.

Gillot, M. 1990. Phylum cryptophyta (cryptomonads). In *Handbook of protoctista.* L. Margulis, J.O. Corliss, M. Melkonian, and D.J. Chapman, eds. Boston: Jones & Bartlett Publishers.

Gillott, M.A., and S.P. Gibbs 1980. The cryptomonad nucleomorph: Its ultrastructure and evolutionary significance. *Jour. Phycol.*, 16:558–568.

Grim, J.N., and L.A. Staehelin. 1984. The ejectisomes of the flagellate *Chilomonas paramecium*: Visualization by freeze-fracture and isolation techniques. *Jour. Protozool.*, 3:259–267.

Guillard, R.R.L. 1975. Culture of phytoplankton for feeding marine invertebrates. In *Culture of marine invertebrate animals.* W.L. Smith and M.H. Chanley, eds. New York: Plenum Press.

Halevy, S., R. Saliternik, and L. Avivi. 1971. Isolation of rhodamine-positive toxins from *Ochromonas* and other algae. *Int. Jour. Biochem.*, 2:185–192.

Hill, D.R.A. 1990. *Chroomonas* and other blue-green cryptomonads. *Jour. Phycol.*, 26:133–145.

Hill, D.R.A. 1991a. Diversity of heterotrophic cryptomonads. In *The biology of free-living heterotrophic flagellates.* The Systematics Association Special Volume 45. D.J. Patterson and J. Larsen, eds. Oxford: Clarendon Press.

Hill, D.R.A. 1991b. A revised circumscription of *Cryptomonas* (Cryptophyceae) based on examination of Australian strains. *Phycologia*, 30:170–188.

Hill, D.R.A., and K.S. Rowan. 1989. Biliproteins of the Cryptophyceae. *Phycologia*, 28:455–463.

Hill, D.R.A., and R. Wetherbee. 1986. *Proteomonas sulcata* gen. et sp. nov. (Cryptophyceae) a cryptomonad with two morphologically distinct and alternating forms. *Phycologia*, 27:521–543.

Hill, D.R.A., and R. Wetherbee. 1988. The structure and taxonomy of *Rhinomonas pauca* gen. et sp. nov. (Cryptophyceae). *Phycologia*, 27:355–365.

Hill, D.R.A., and R. Wetherbee. 1989. A reappraisal of the genus *Rhodomonas* (Cryptophyceae). *Phycologia*, 28:143–158.

Hoef-Emden, K., and M. Melkonian. 2003. Revision of the genus *Cryptomonas* (Cryptophyceae): A combination of molecular phylogeny and morphology provides insights into a long-hidden dimorphism. *Protist*, 154:371–409.

Klaveness, D. 1981. *Rhodomonas lacustris* (Pascher and Ruttner) Javornicky (Cryptomonadida): Ultrastructure of the vegetative cell. *Jour. Protozool.*, 28:83–90.

Klaveness, D. 1984. Studies on the morphology, food selection and growth of two planktonic freshwater strains of *Coleps* sp. *Protistologica*, 20:335–349.

Klaveness, D. 1985. Classical and modern criteria for determining species of Cryptophyceae. *Bull. Plankton Soc. Jpn.*, 32:111–128.

Klaveness, D. 1988. Ecology of the Cryptomonadida: A first review. In *Growth and reproductive strategies of freshwater phytoplankton.* C.D. Sandgren, ed. New York: Cambridge University Press.

Kugrens, P., and B.L. Clay. 2003. Cryptomonads. In *Freshwater algae of North America: Ecology and classification.* J.D. Wehr and R.G. Sheath, eds. Amsterdam, Boston: Academic Press. pp. 715–756.

Kugrens, P., and R.E. Lee. 1987. An ultrastructural survey of cryptomonad periplasts using quick-freezing freeze-fracture techniques. *Jour. Phycol.*, 23:365–376.

Kugrens, P., and R.E. Lee. 1988. Ultrastructure of fertilization in a cryptomonad. *Jour. Phycol.*, 24:510–518.

Kugrens, P., and R.E. Lee. 1991. Organization of cryptomonads. In *The biology of free-living heterotrophic flagellates.* The Systematics Association Special Volume 45. D.J. Patterson and J. Larsen, eds. Oxford: Clarendon Press.

Kugrens, P., R.E. Lee, and R.E. Andersen. 1986. Cell form and surface patterns in *Chroomonas* and *Cryptomonas* cells (Cryptophyta)

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as revealed by scanning electron microscopy. *Jour. Phycol.*, 22:512–522.

Kugrens, P., R.E. Lee, and R.E. Andersen. 1987. Ultrastructural variations in cryptomonad flagella. *Jour. Phycol.*, 23:511–518.

Kugrens, P., R.E. Lee, and J.O. Corliss. 1994. Ultrastructure, function and biogenesis of extrusive organelles in selected non-ciliate protists. *Protoplasma*, 181:164–190.

Kugrens, P., B.L. Clay, and R.E. Lee. 1999. Ultrastructure and systematics of two new freshwater cryptomonads, *Pyrenomonas ovalis* and *Storeatula rhinosa* sp. nov. *Jour. Phycol.*, 35:1079–1089.

Lane, C.E., K. van den Heuvel, C. Kozera, B.A. Curtis, B.J. Parsons, S. Bowman, and J.M. Archibald. 2007. Nucleomorph genome of *Hemiselmis andersenii* reveals complete intron loss and compaction as a driver of protein structure and function. *PNAS*, 104:19908–19913.

Lee, R.E., and P. Kugrens. 1991a. *Katablepharis ovalis*, a colorless flagellate with interesting cytological characteristics. *Jour. Phycol.*, 27:505–513.

Lee, R.E., and P. Kugrens. 1991b. Attachment strips: A new type of hemidesmosome-like structure in the protozoon *Katablepharis ovalis* Skuja. *Jour. Cell Sci.*, 98:245–249.

Lee, R.E., P. Kugrens, and A.P. Mylnikov. 1991. Feeding apparatus of the colorless flagellate *Katablepharis* (Cryptophyceae). *Jour. Phycol.*, 27:725–733.

Lewitus, A.J., H.B. Glasgow, and J.M. Burkholder. 1999. Kleptoplastidy in the toxic dinoflagellate *Pfiesteria piscicida* (Dinophyceae). *Jour. Phycol.*, 35:303–312.

Li, A., D.K. Stoecker, D.W. Coats, and J. Adam. 1996. Ingestion of fluorescently labeled and phycoerythrin-containing prey by photosynthetic dinoflagellates. *Aquat. Microb.* Ecol. 10: 139–147.

Ludwig, M., and S.P. Gibbs. 1985. DNA is present in the nucleomorph of cryptomonads: Further evidence that the chloroplast evolved from a eukaryotic endosymbiont. *Protoplasma*, 127:9–20.

McFadden, G.I. 1990. Evidence that cryptomonad chloroplasts evolved from photosynthetic eukaryotic endosymbionts. *Jour. Cell Sci.*, 95:303–308.

Meyer, S.R., and R.N. Pienaar. 1984. The microanatomy of *Chroomonas africana* sp. nov. (Cryptophyceae). *S. Afr. Tydskr. Plankton*, 3:306–319.

Mignot, J.P. 1965. Etude ultrastructurale de *Cyathomonas truncata* From. (Flagelle Cryptomonadine). *Jour. Microsc.*, 4:239–252.

Morgan, K., and J. Kalff. 1975. The winter dark survival of an algal flagellate *Cryptomonas erosa* (Skuja). *Verh. Int. Verein. Limnol.*, 19:2735–2740.

Morgan, K.C., and J. Kalff. 1979. Effect of light and temperature interactions on growth of *Cryptomonas erosa* (Cryptophyceae). *Jour. Phycol.*, 15:127–134.

Munawar, M., and T. Bistricki. 1979. Scanning electron microscopy of some nanoplankton cryptomonads. *Scanning Electr. Microsc.*, 3:247–252.

Novarino, G. 1993. Possible detection of the periplast areas and the nucleomorph of cryptomonads by light microscopy: Some early observations by Künstler, Skuja and Hoolande. *Quekett Jour. Microsc.*, 37:45–51.

Novarino, G., I.A.N. Lucas, and S. Morrall. 1994. Observations on the genus *Plagioselmis* (Cryptophyceae). *Cryptogamie Algologie*, 15:87–96.

Okamoto, N., and I. Inouye. 2005. The katablepharids are a distant sister group of the Cryptophyta: A proposal for Katablepharidophyta division nova. *Protist*, 156:163–179.

Pejler, B. 1977. Experience with rotifer cultures based on *Rhodomonas*. *Arch. Hydrobiol. Beih. Ergebn. Limnol.*, 8:264–266.

Pennick, N. 1981. Flagellar scales in *Hemiselmis brunnescens* Butcher and *H. virescens* Droop (Cryptophyceae). *Archiv Protisten.*, 124:267–270.

Pennick, N. 1982. Observations on the fine structure of *Hemiselmis brunnescens* Butcher. *Arch. Protisten.*, 126:241–245.

Pfiester, L.A., and J.R. Holt. 1978. A freshwater "red tide" in Texas. *Southwestern Nat.*, 23:103–110.

Pringsheim, E.G. 1968. Zur Kenntnis der Cryptomonaden des Süsswassers. *Nova Hedw.*, 16:367–401.

Roberts, K.R. 1984. Structure and significance of the cryptomonad flagellar appartus. I. *Cryptomonas ovata* (Cryptophyta). *Jour. Phycol.*, 20:590–599.

Santore, U.J. 1977. Scanning electron microscopy and comparative micromorphology of the periplast of *Hemiselmis rufescens, Chroomonas* sp., *Chroomonas salina* and members of the genus *Cryptomonas* (Cryptophyceae). *Eur. Jour. Phycol.*, 12:255–270.

Santore, U.J. 1978. Light- and electron-microscopic observations of the palmelloid phase in members of the genus *Cryptomonas* (Cryptophyceae). *Arch. Protisten.*, 120:429–435.

Santore, U.J. 1984. Some aspects of taxonomy in the Cryptophyceae. *New Phytol.*, 98:627–646.

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Sarnelle, O. 1993. Herbivore effects on phytoplankton succession in a eutrophic lake. *Ecol. Monogr.*, 63:129–149.

Stemberger, R.S., and J.J. Gilbert. 1985. Body size, food concentration, and population growth in planktonic rotifers. *Ecology*, 66:1151–1159.

Tranvik, L.J., K.G. Porter, and J. Sieburth. 1989. Occurrence of bacterivory in *Cryptomonas*, a common freshwater phytoplankter. *Oecologia*, 78:473–476.

Wetherbee, R., D.R.A. Hill, and G.I. McFadden. 1986. Periplast structure of the cryptomonad flagellate *Hemiselmis brunnescens*. *Protoplasma*, 131:11–22.

Wetherbee, R., D.R.A. Hill, and S.J. Brett. 1987. The structure of the periplast components and their association with the plasma membrane in a cryptomonad flagellate. *Can. Jour. Bot.*, 65:1019–1026.

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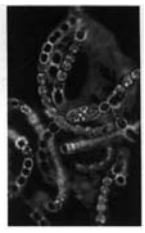

Chapter 10

Bacillariophyta: The Diatoms

Joshua T. Cooper, Miriam Steinitz Kannan, and Diane E. Kallmeyer

GENERAL INTRODUCTION TO THE DIATOMS

Biology

Diatoms are unicellular, colonial, or filamentous algae characterized by having golden-brown chloroplasts and cell walls made of silica. The cell wall is often surrounded by polysaccharide mucilage that may extend to form stalks used for the cells to attach to surfaces. The International Society of Protistologists classifies diatoms as Stramenopiles within the supergroup Chromalveolata (Adl et al. 2005), a major line in the domain Eukarya. Diatom taxonomy is largely based on the structural features of the cell wall. The cell wall (called a frustule) consists of two overlapping halves, like the top and bottom of a box. They are called the epitheca and the hypotheca, respectively. Like a box, they appear different if viewed from the top or bottom (the epivalve or hypovalve) or from the sides (girdle view). The girdle (or cingula) that connects the two valves can be simple closed or open bands, or consist of several sections called girdle bands (Figure 10-1).

Diatoms come in two basic forms: centric (radially symmetrical) and pennate (not radially symmetrical). They have pores on the valve and girdle that are called, depending on size, areolae, loculae, poroids, or punctae. Rows of punctae are called striae and often appear as lines when viewed under a light microscope. Pennate diatoms may have parallel hollow chambers called costae. One of the most important cell wall structures for diatom classification is the raphe. The raphe consists of longitudinal fissures involved in motility. It is only found in pennate diatoms. The raphe in some genera is present in only one valve, and a clear area called the pseudoraphe is seen in its place on the other valve. The raphe occurs at the center of the valve in some genera, interrupted by a central nodule. In other genera it is found at the edge, sometimes raised in a keel structure or enclosed in a canal. Diatoms that lack a raphe

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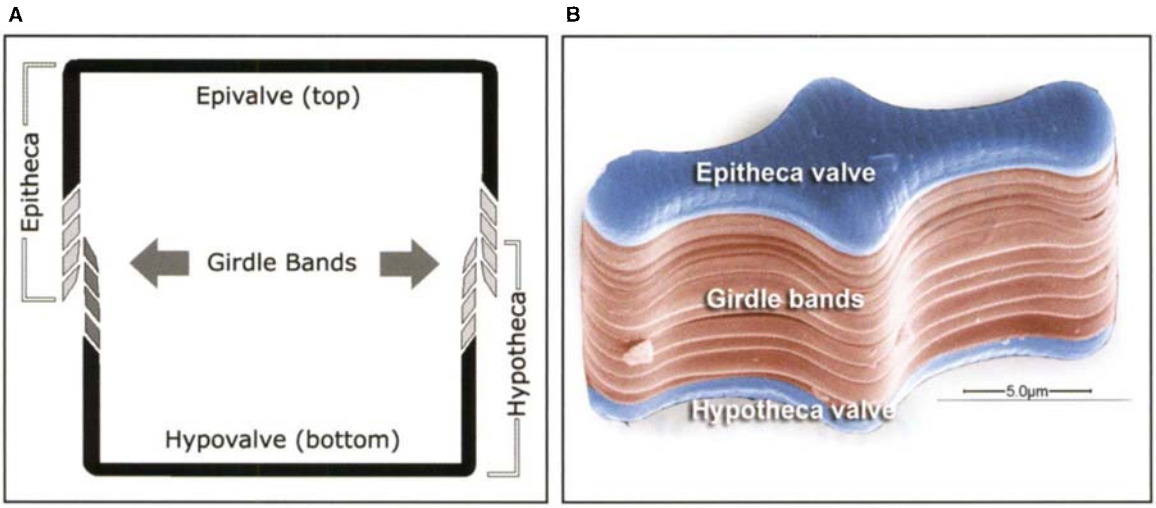

Figure 10-1 (A) Cross-section through a diatom cell, showing the two halves of the box (frustule). The epivalve (top) and hypovalve (bottom) are connected via girdle bands attached to them, respectively. The epitheca refers to the epivalve and attached girdle bands, and the hypotheca, to the hypovalve with attached girdle bands. (B) scanning electron microscope photograph of *Tabellaria flocculosa* showing these features.

(araphid) may have tubular structures called rimoportula that end with an expanded "lip" with a slit called labiate process involved in the excretion of mucilage. The rimoportula may extend externally and appear as a spine. Some centric diatoms excrete the mucilage though a large pore, the ocellus. The Thalassiosirales have tubular structures called strutted processes, through which they secrete chitin fibrils that unite cells in short chains. Figure 10-2 shows many of the features described above. More details on the cell wall structure are given in Round et al. 1990.

Structure of living cells. Diatoms have the typical structures of eukaryotic cells with a single nucleus that is either round or kidney-shaped. Flagella are present only in the gametes. The golden-brown chloroplasts contain chlorophyll *a*, chlorophyll *c*, fucoxanthin, β-carotene, ε-carotene, diatoxanthin, and diadinoxanthin. The golden-brown coloration is due to the carotenes and the fucoxanthin. The chloroplasts show considerable variation in shape and arrangement (Mann 1996). Most centric diatoms have numerous small rounded chloroplasts, while pennates have fewer (one or two) large ribbonlike chloroplasts. Chloroplasts cluster around the nucleus when illumination is strong, protecting the DNA from UV radiation damage. The storage products of photosynthesis in diatoms are oils, mannitol, and chrysolaminarin. Figure 10-3 shows typical diatom chloroplasts.

Reproduction and cell wall formation. Detailed images of live diatom cells are portrayed in a film by Pickett-Heaps and Pickett-Heaps (2003). The film describes their unique mitotic cell division, cell wall formation, and motility. During cell division, hypovalves are usually formed inside the frustule, and as a result successive generations will be smaller than the parent cells. When diatom populations reach a critical small size, sexual reproduction is initiated and the resulting zygote forms a dormant auxospore. The auxospores eventually produce, by mitosis, two large diploid cells restoring the largest cell size of the life cycle of a given species. It is very rare to see either sexual reproduction or auxospores in nature (Round et al. 1990). There are species where this size reduction may not occur. *Melosira* cells have been observed in culture where the new hypovalve expands to the size of the parent cell after division. Recent research on morphogenesis of the silica cell wall during cell division describes silica deposition involving enzymes known as silifins and silica transport channels

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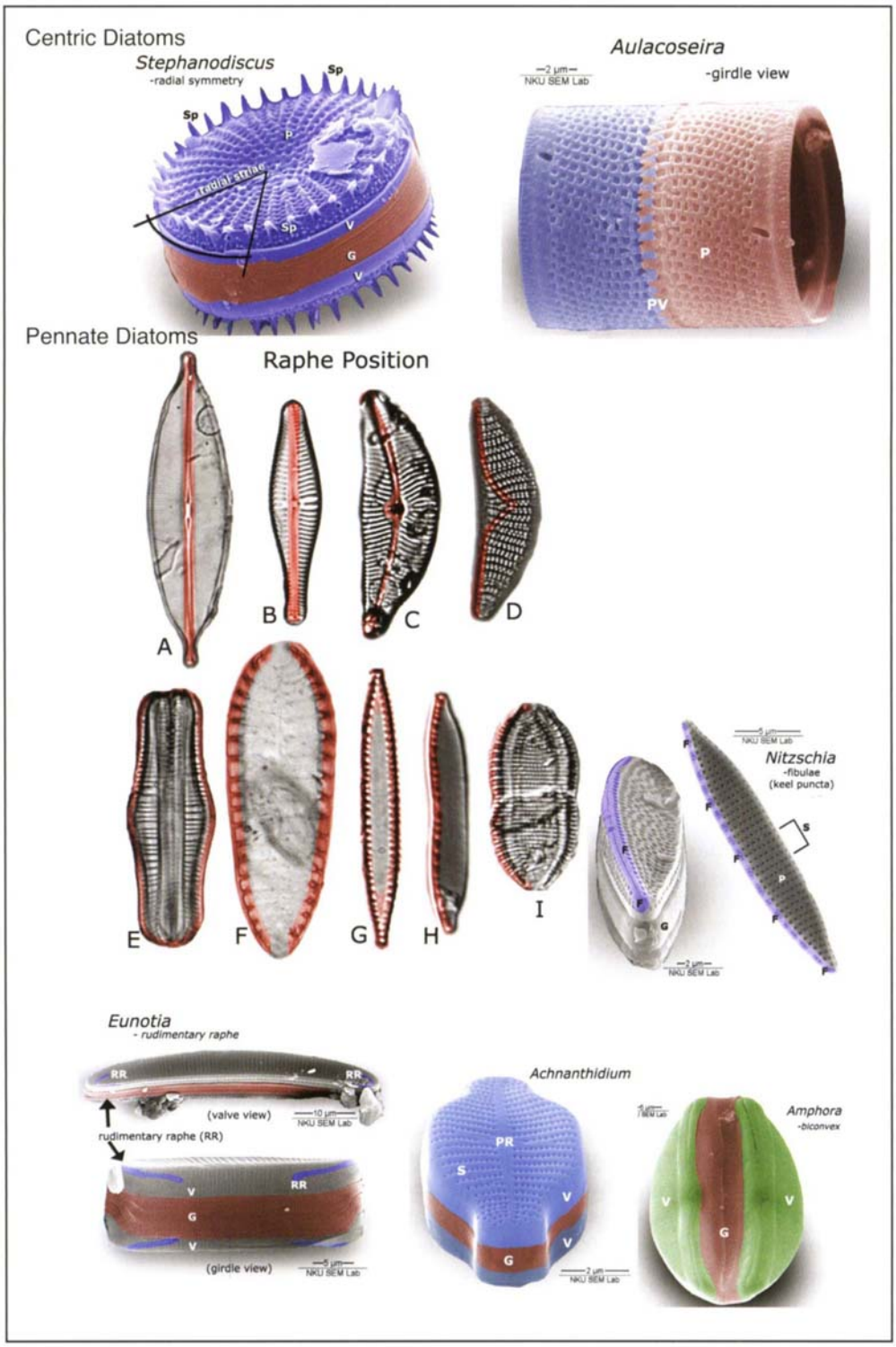

Figure 10-2 Photomicrographs of diatoms showing many of the cell wall features. *Stephanodiscus* and *Aulacoseira* are centric diatoms. All diatoms with a raphe or pseudoraphe are pennate. The position of the raphe is shown in color. The raphe can be in the center of the valve (A, B, C), enclosed in a canal (D, E), on a "wing" (F), or on a keel (G, H, I, *Nitzschia*). *Eunotia* shows a rudimentary raphe. *Achnanthidium* has a raphe on one valve and a pseudoraphe on the other. In *Amphora*, the raphe can be seen at the same time in the two valves (v). Other features include V = valve, G = girdle, PV = pervalvar axis, s = striae, P = punctae, Sp = spines, F = fibulae, RR = rudimentary raphe.

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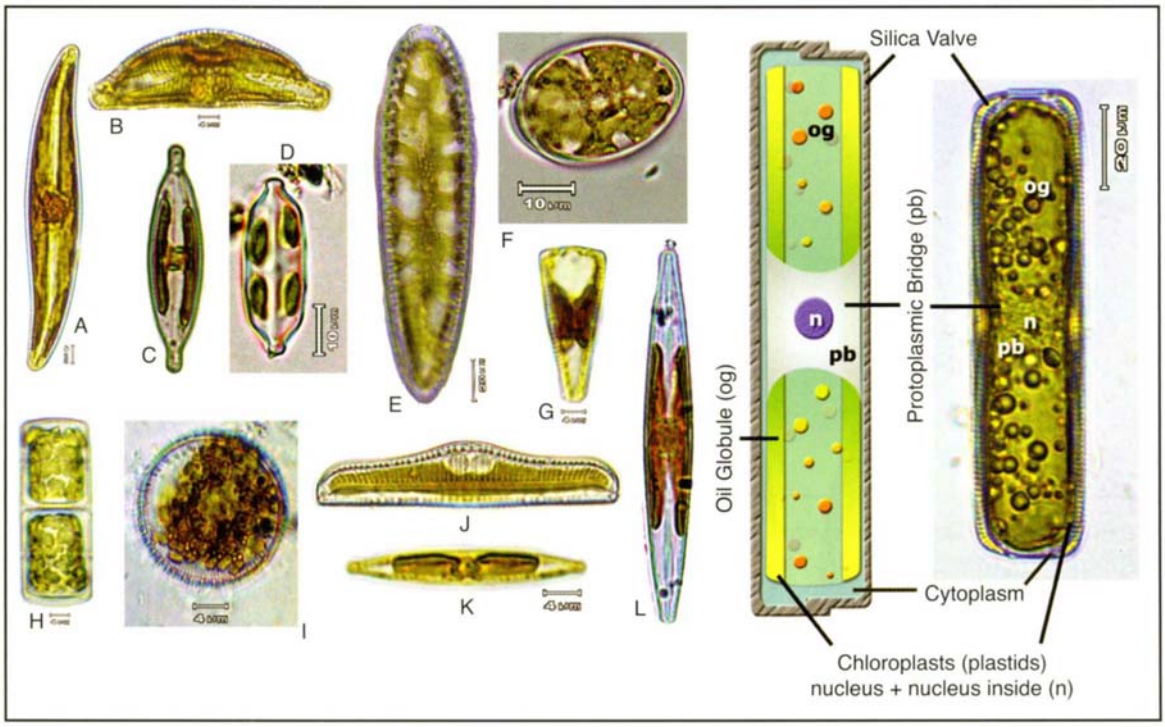

Figure 10-3 Structure of living diatom cells (right) and chloroplasts (plastids): (A) *Gyrosigma*: Plastid is marginal and wavy and restricted to the sides; (B) *Cymbella*; (C, L) *Navicula* and *Amphipleura* have H-shaped plastids; (D) *Neidium* has four irregularly placed plastids in four quads, separated by the raphe and the cytoplasmic bridge; (E, F) *Surirella* and *Cocconeis* show undulating chloroplast shapes; (G) *Gomphonema* shows the plastid in girdle view; (H, I) *Melosira* and *Stephanodiscus* have numerous elliptical to spheroid plastids typical of centric and araphid diatoms; (J) *Rhopalodia* has a large, marginal plastid; and (K) *Nitzschia* has two plastids that are separated by the cytoplasmic bridge between the valves.

that actively transport and help to concentrate orthosilicic acid from the environment (Schmid 1979, Li and Volcani 1984, Thamatrakoln and Hildebrand 2007).

Diatom motility and mucilage secretions. Diatoms move by exuding mucilaginous fluid through the raphe. The fluid is left behind as a trail as the diatoms glide over it (Gordon and Drum 1970). The polysaccharide mucilage that forms the "raphe-fluid" appears to have the same chemical composition as the mucilage involved in stalk production or any general mucilage that allows diatoms to adhere to surfaces (Wang et al. 1997). Recent studies indicate that some diatoms that do not contain a raphe are still able to move. They secrete this mucilage with force through the labiated process and propel themselves like tiny "rockets," leaving the mucilage behind (Pickett-Heaps and Pickett-Heaps 2003). Another mechanism for motility is as mucilage is secreted; it swells by uptake of water and pushes the cell along.

One of the most striking diatoms, when it comes to its motility, is *Bacillaria paradoxa*, which is capable of performing a "dance" as cells in a colony glide past each other, going from lining up end to end into very long filaments to lining side to side (Ussing et al. 2005). Such dance probably has a selective advantage as it lets each cell in a *Bacillaria* colony be present in a different point in the water column, allowing it to exploit a "new" supply of nutrients. This movement also allows the entire colony of *Bacillaria* to remain suspended in the plankton for longer periods of time (somewhat like swimming). Planktonic diatoms can also control their position in the water column by the production of oils and by gas vacuoles filled with oxygen from photosynthesis

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that allow the cells to float or sink. Some centric diatoms are planktonic because they secrete beta-chitinous fibers, instead of mucilage, that connect cells together to form a "net" and increase surface area.

Ecology

Diatoms are found in any habitat that is wet, including marine, brackish, freshwater, and any permanently moist surface. The various diatom microhabitats are summarized in Figure 10-4. The majority of species are benthic (i.e., found in the bottom of lakes, streams, or rivers) or periphytic (i.e., found in shallow or littoral areas). Microhabitats within the benthic and periphyton group include epiphytic (on plants), epipsammic (attached to sand), epipelic (on mud), or epilithic (on rocks). Some species are epizootic (attached to animals such as turtles or snails). Planktonic species of diatoms live in the open water column and have adaptations for buoyancy. Generally, planktonic species are without a raphe system (araphinous). Many have spines or can form stellate or filamentous colonies to increase surface area, and some use the vacuole/oil droplets to control buoyancy. They are considered euplanktonic when free-floating and live their entire lives suspended in the water column, or tychoplanktonic when they live in the shallows or the bottom but become suspended in the water column and therefore are collected in the "plankton." Tychoplanktonic diatoms require turbulent waters in order to stay suspended within the photic zone. Aerophilic species of diatoms inhabit damp places also in association with other algae. They are also common in moist, terrestrial habitats like damp soil and beneath waterfalls. They have lightly constructed frustules that allow for them to be transported by the atmosphere in water droplets associated with evaporation and can fall back down with precipitation.

Diatoms are particularly useful water quality indicators because they are ubiquitously distributed, are naturally high in the number of species, and show a wide range of tolerance to pollution. The tolerance or sensitivity with respect to nutrients, pH, salinity, degree of pollution, eutrophication, and specific changes in environmental conditions are known for many species. (Stevenson and Pan 1999, Van Dam et al. 1994, Lowe 1974). Diatoms also have a very short generation time (approximately two weeks) for the population to double itself, thus communities rapidly change in response to environmental changes. Diatoms with deformed valves can occur when dissolved heavy metal concentrations such as copper or mercury are high (Steinitz-Kannan 2005, Dickman 1998, Ruggiu et al. 1998, Dickman et al. 1990). The presence of diatom species that tolerate more acidic conditions (lower pH) and high sedimentation suggest influences of land usage such as acid mine drainage (Camburn and Charles 2000, Verb and Vis 2000, Smol et al. 1986). Diatoms are also sensitive to variations in available light, moisture conditions (some tolerate drought), temperature, and current velocity. The use of diatoms as indicators is particularly well developed by paleoecologists who use fossil diatoms to indicate past lake levels, change in land use, trophic conditions, pH, and salinity (Ruggiu et al. 1998, Dixit et al. 1992, Fritz et al. 1991, Battarbee 1986).

Table 10-1 lists the most common freshwater genera of diatoms and the ecological conditions that favor that particular genus. The information is taken from the available literature (Verb and Vis 2000; Van Dam et al. 1994; Lange-Bertalot and Moser 1994; Krammer and Lange-Bertalot 1986, 1988a, 1988b; Lowe 1974; Lobo 1997; Patrick 1963; Patrick and Reimer 1966, 1975; Palmer 1962, 1977; and others cited in the table) as well as information held at the Northern Kentucky University's Diatom Herbarium collection. There are genera and species that are tolerant of a wide range of environmental conditions and others whose tolerance is narrower. Therefore it is more accurate to look at the entire diatom community and not to judge water quality based

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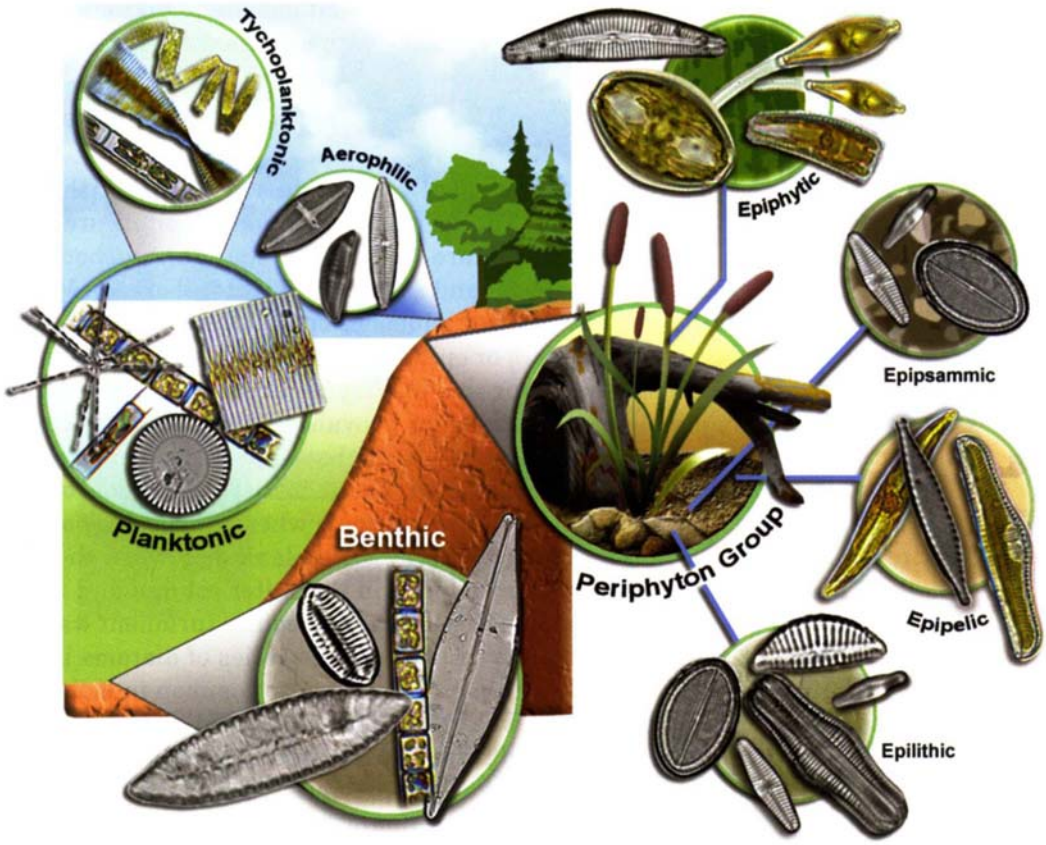

Examples shown: planktonic (*Asterionella, Aulacoseira, Fragilaria, Melosira, Cyclotella*); tychoplanktonic (*Aulacoseira, Fragilaria, Tabellaria*); aerophilic (*Luticola, Navicula cincta, Amphora pediculus*); periphyton: epiphytic (*Cymbella, Amphora, Gomphonema, Rhoicosphenia*); epipsammic (*Achnanthidium, Planothidium, Cocconeis*); epipelic (*Gyrosigma, Nitzschia, Rhopalodia*); epilithic (*Cocconeis, Cymbella, Achnanthidium, Rhopalodia, Planothidium*); and benthic (*Surirella, Tribionella, Melosira, Craticula*).

Figure 10-4 Different ecological microhabitats for diatoms in various ecosystems. Diatom genera are adapted to their respective microhabitats using specialized features of the siliceous cell wall.

just on the presence or absence of a single genus or species. Ideally, when describing a diatom community, diatoms should be identified to the species level, then, with the use of transfer functions, estimations of variables such as salinity, pH, or trophic level of a body of water can be made with greater accuracy (Dixit et al. 1992, Fritz et al. 1991), but that is beyond the objectives of this manual.

SIGNIFICANCE OF DIATOMS TO THE DRINKING WATER INDUSTRY

Source Water

Diatoms are likely to be the most abundant species in the source water for drinking water utilities. Utilities that use the Ohio River as source water, for example, report *Aulacoseira* usually as the dominant species, particularly in summer (ORSANCO personal communication). The most important reason to test the source water for diatoms is because they are outstanding indicators of water quality (See Ecology section.).

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Table 10-1 Summary of ecological information for freshwater diatom genera

Genus	Figure	Habitat, Distribution, and Comments
Acanthoceras	10-6	Planktonic in large rivers.
Achnanthes, *Achnanthidium,* *Planothidium,* *Psammothidium,* *Rossithidium*	10-30 10-27 10-28 10-29	Widely distributed in various habitats such as aerophilic, benthic, periphytic, epiphytic, and epipsammic. They are primarily colonizers on biofilms in streams (Stevenson et al. 1996). They connect to substrates by mucilaginous stalks. NOTE: Old taxonomic keys describe all these genera as *Achnanthes.* These new genera represent freshwater taxa, while *Achnanthes* is still used to describe marine taxa.
Actinella	10-31	Soft, acidic waters (Kociolek and Spaulding 2003b), solitary or in small colonies enclosed in mucilage. Benthic.
Amphora	10-53	Common benthic epiphytic diatom often growing in streams attached to surfaces like aquatic plants or other algae. The genus is very tolerant of high salinity, and most species within the genera are marine or found in hypersaline environments. Cells are solitary, attached by mucilaginous stalks or found in mucilaginous tubes (Cox 1996).
Amphipleura	10-61	Widely distributed in standing or slow-moving, alkaline waters. Usually solitary but may be colonial, enclosed in gelatinous tubes (Patrick and Reimer 1966).
Anomoeoneis	10-57	Epipelic in shallow, high-conductivity, and brackish waters. Some species within this genus tolerate high levels of organic pollution and low oxygen (Cox 1996, Lange-Bertalot and Moser 1994, Kociolek and Spaulding 2003a). NOTE: Round and Mann split this group into *Anomoeoneis, Nupela,* and *Brachysira. Brachysira* is found in soft, acidic waters.
Asterionella	10-20	Planktonic in lakes and large rivers. It requires relatively high nutrient levels. Listed as both taste and odor causing and filter clogging (APHA et al. 2005).
Aulacoseira	10-5	Tychoplanktonic in freshwater lakes, ponds, and large rivers. Some species survive sediment burial for extended periods of time. Their presence in the plankton of lakes indicates mixing. NOTE: Old taxonomic keys lump the genus *Aulacoseira* within the genus *Melosira* (Round et al. 1990).
Bacillaria	10-33	Form-linked colonies attached at the raphe slits that move by gliding against each other. Colonies can be epipelic or tychoplanktonic (Lowe 2003). Found in water with high levels of dissolved solids, including brackish waters.
Brachysira	10-69	Common in acidic, low-conductivity freshwaters. It is found in dystrophic or oligotrophic lakes, swamps, or bogs, where it grows attached to the substrate by mucilaginous stalks, or it can be solitary and tychoplanktonic. NOTE: The genus was originally lumped under *Anomoneis*, and was split by Round and Mann (1981) into *Brachysira, Nupela*, and *Anomoneis.*
Caloneis	10-65	Benthic. More prevalent in acidic habitats with low conductivity.
Campylodiscus	10-35	Most species are restricted to high conductivity or saline environments; a few are found in freshwaters in benthic lake ecosystems. The most common freshwater species in North America is *C. noricus* (Lowe 2003).
Capartogramma	10-63	Found in flowing waters (rheophilic) in the United States, of which only one species is reported, *C. crucicula* (Kociolek and Spaulding 2003a).

Table continued next page

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Table 10-1 Summary of ecological information for freshwater diatom genera (continued)

Genus	Figure	Habitat, Distribution, and Comments
Cocconeis	10-26	Cells are adapted to live on plants or other substrates (epiphytic) and are usually found in large numbers but live as solitary cells. Some species within the genus occur in waters of moderate to high electrolyte content, to brackish waters. They can tolerate meso- to eutrophic, flowing or still waters. *Cocconeis* are sometimes considered a primary colonizer and "understory" taxa to an attached diatom community.
Coscinodiscus		Planktonic but there is much taxonomic confusion about this genus. Most species found in freshwaters have been found to belong to *Thalassiosira.* Better optics has revealed previously unnoticed spines, processes, or ocelli, causing species to be renamed under other genera. Due to current taxonomic confusions, ecology is under evaluation.
Craticula	10-68	Found in electrolyte-rich and brackish waters. The genus is resistant to pollution and is found in mesotrophic to eutrophic habitats (Cox 1996).
Cyclostephanos	10-11	Considered indicators of eutrophication; spring and summer dominant taxa in shallow, eutrophic lakes and large eutrophic rivers (Stoermer and Julius 2003).
Cyclotella	10-10	Widely distributed in open water habitats from lakes to large rivers. Environmental requirements are species specific. Most species are planktonic and solitary. Some species create a mass of organic mucilage in which cells can be connected into loosely filamentous colonies or floating within the mucilage. These can potentially clog filters.
Cymatopleura	10-36	Benthic diatom common in nutrient-rich waters.
Cymbella	10-54	Cells grow in benthic habitats surrounded in mucilage or attached to substrate with mucilaginous stalks. These can potentially clog filters. NOTE: See also *Encyonema,* which was previously lumped with *Cymbella.*
Denticula	10-40	Found on rocks (benthic) in fresh to brackish waters (Van Heurck 1896). They form short chains attached at the girdle face of the valve or are solitary.
Diadesmis	10-70	Aerophilic, found in aquatic and terrestrial habitats attached to damp limestone cliffs or rocks, standing waters, and springs (of low conductance and slightly acidic waters) and also found on earth/soil, on moss, in greenhouses, and under low-light conditions, as in caves (Kociolek and Spaulding 2003a, Cox 1996).
Diatoma	10-18	Tychoplanktonic or epiphytic in slow-moving rivers, springs, and upland flowing waters (rheophilic) of higher electrolyte content. Also often found in ponds and the littoral zone of lakes. Tolerates moderate to high nutrient enrichment. Live cells join to form ribbonlike or stellate (star-shaped), zigzag colonies. Potential filter clogging.
Didymosphenia	10-51b	Originally restricted to northern latitudes in cool, low-nutrient waters. Now found in streams and rivers in much of North America, it is considered an invasive species and poses a threat because it forms extensive mats on streambeds. It attaches to rocks by stalks that have a rough texture similar to wet wool. Only one species is reported in North America, *D. geminata.*
Diploneis	10-59	Found in oligotrophic waters low in organic matter that are standing or well aerated. Electrolite requirements vary with the species.

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Table 10-1 Summary of ecological information for freshwater diatom genera (continued)

Genus	Figure	Habitat, Distribution, and Comments
Encyonema	10-55	Epiphytic, solitary, attached to a substrate or colonial enclosed in mucilage tubes. Found in waters that vary from oligotrophic to eutrophic, some are pollution tolerant (Cox 1996). NOTE: *Encyonema* was previously lumped into *Cymbella* but has been separated based on ultrastructural morphology. *Cymbella* is listed as potential filter clogging by APHA et al. 2005.
Entomoneis	10-37	Epipelic (benthic) in water of high conductivity (brackish), in some cases considered tychoplanktonic.
Epithemia	10-44	Benthic species in hard water habitats where phosphorus is more available than nitrogen. Common on the surface of submerged aquatic plants (epiphytic). NOTE: Species contain endosymbiotic cyanobacteria that function in nitrogen fixation. This allows the genus to exist in low-nitrogen/phosphorus microhabitats (Prechtl et al. 2004).
Eunotia	10-32	Characteristic of low conductivity, acidic waters. It is often present in oligotrophic or dystrophic lakes or bogs. Live cells form ribbonlike colonies attached at the rudimentary raphe or by mucilaginous pads. Some are solitary or found at the ends of short stalks.
Fragilaria	10-24	Under the old taxonomy, *Fragilaria* was distinguished from *Synedra* because it formed long, ribbonlike chains and was usually planktonic, unlike *Synedra,* which was usually solitary and attached. Taxonomy is currently under debate and some authors have merged *Synedra* under *Fragilaria. Fragilaria is* listed as potential filter clogging by APHA et al. 2005.
Frustulia	10-60	Often abundant and usually characteristic of acidic water with very low conductivity. They can be benthic, aerophilic, or tychoplanktonic. NOTE: *Frustulia* is often found in association with *Brachysira.*
Geissleria	10-71b	Broadly distributed across North America and can be found in oligotrophic to eutrophic waters (Kociolek and Spaulding 2003a). NOTE: *Geissleria* was previously classified under *Navicula.*
Gomphoneis	10-50	Found attached to substrate by mucilaginous stalks. Cells grow upward from the stalks like a flower, connected at the narrow elongate point of the frustule. Large groupings of attached *Gomphoneis* form thick, mucilaginous colonies on the edges of rivers, lakes, and streams (Kociolek and Spaulding 2003b).
Gomphonema	10-51a	Epiphytic genus found in shallow water in nearly every habitat. Grows at ends of mucilaginous stalks or forms stellate colonies or mucilaginous masses.
Gyrosigma	10-48	Rheophilic. Found in high-conductivity freshwater to brackish water. Though present in flowing and standing waters, it is more common in fast-running streams and rivers because of its high oxygen requirements (Cox 1996).
Hannaea	10-19	Benthic genus common in mountain streams worldwide and in large, cold lakes (Lake Superior, Lake Baikal). Seems to prefer cold, oligotrophic waters of low conductance and moderate acidity.
Hantzschia	10-42	Benthic species found in a wide range of habitats. Can tolerate periodic drying (aerophilic). Common in freshwaters with high conductivity, brackish water, moist soil, and moist rock crevices.

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Table 10-1 Summary of ecological information for freshwater diatom genera (continued)

Genus	Figure	Habitat, Distribution, and Comments
Hippodonta	10-71d	Characteristic of benthic habitats, tolerates high salinity levels, and is typically found in lakes (Kociolek and Spaulding 2003a). NOTE: *Hippodonta* was previously classified under *Navicula.*
Luticola	10-56	Characteristic of soil, aerophilic habitats, and moss habitats (Kociolek and Spaulding 2003a). *Luticola* is also common in brackish to freshwaters prone to drying out and on damp surfaces; some are tolerant to very heavy pollution (Cox 1996). NOTE: *Luticola* previously was classified under *Navicula* .
Martyana	10-21	Characteristic of sandy habitats (epipsammic) and produces mucilaginous pads at the narrow end of the valve (Kingston 2003, Cox 1996). NOTE: *Martyana* was previously classified under *Opephora* and is now restricted to freshwater taxa.
Mastogloia	10-58	Benthic in highly alkaline waters and marl lakes (high in calcium carbonate). Most species are marine, and only a few are actually found in freshwaters (Kociolek and Spaulding 2003a).
Melosira	10-8	*Melosira varians* is common in the plankton of eutrophic rivers and lakes. Most other species are marine.
Meridion	10-17	Occurs in upland waters of low conductivity or can be found in calcium-rich springs and streams particularly in colder waters (Cox 1996). Occasionally found in eutrophic rivers.
Navicula	10-71a	Found in almost every freshwater habitat and environment, they indicate the presence of water. Many species can be pollution tolerant, and some could be sensitive to forms of pollution. NOTE: This genus is a very large group that has many subgenera and recently has been split into many genera (e.g., *Luticola*, *Placoneis*, *Sellaphora*, and *Craticula*). Listed as potentially filter clogging by APHA et al. 2005.
Neidium	10-62	Found in oligotrophic, low-organic matter to dystrophic waters of low to moderate conductivity. Often prevalent in acidic waters.
Nitzschia	10-43	Most species of *Nitzschia* are nitrogen heterotrophs and are present in waters rich in organic matter and of high BOD (biochemical oxygen demand); therefore they can be organic pollution indicators. Some species are potentially toxic (Rangel et al. 1997).
Pinnularia	10-66	Benthic, found in a variety of habitats. Many species tolerate acidic waters.
Placoneis	10-71c	Found primarily in freshwaters, occurring in epipelic habitats of lakes and streams. Found in widespread ecological conditions, but more common in oligotrophic waters of low to high conductivity. Some species tolerate moderate pollution. (Kociolek and Spaulding, 2003a, Cox 1996). NOTE: It was previously classified under *Navicula.*
Plagiotropis	10-34	Epipelic in brackish waters and freshwater. There are only two freshwater species reported from North America (Kociolek and Spaulding 2003a).
Planothidium	10-28	Very common, epilithic or epiphytic, found in both flowing and standing waters. Live cells attach to any surface by the raphe-valve face or by a mucilaginous pad. It prefers neutral to alkaline waters, is indifferent to conductivity (low to high), and is usually tolerant up to mesotrophic conditions. NOTE: This genus appears as *Achnanthes lanceolata* in older taxonomic literature. *Planothidium* was recently split from the *Achnanthidium* group, which was split from the genus *Achnanthes.*

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Table 10-1 Summary of ecological information for freshwater diatom genera (continued)

Genus	Figure	Habitat, Distribution, and Comments
Pleurosigma	10-49	The genus is primarily found in marine environments, but there are some inland, brackish to high-conductivity freshwater species. They are epipelic and rheophilic.
Pleurosira	10-7	Very common in small streams with high conductivity. They form distinctive zigzag chains that are connected at the ocelli. NOTE: This genus is referred to as *Biddulphia* in older taxonomic works.
Reimeria	10-52	Found on damp rocks, mosses, and other subaerial surfaces (aerophilic).
Rhoicosphenia	10-25	Epiphytic, attached by mucilaginous stalks at the narrow point of the valve. They are pollution tolerant and found abundantly in highly conductive freshwater and brackish waters (Cox 1996). They can be an indicator of salinification of freshwaters due to human disturbance and impact.
Rhopalodia	10-45	Characteristic of hard waters and nitrogen-poor, benthic habitats. NOTE: Usually found in association with *Epithemia* because both tolerate low-nitrogen conditions because they can contain nitrogen-fixing endosymbiotic cyanobacteria (Prechtl et al. 2004).
Rossithidium	10-29	Characteristic of epiphytic to epipsammic microhabitats. Usually found in association with *Achnanthidium minutissimum* (Kingston 2003). NOTE: It was previously classified under *Achnanthes.*
Sellaphora	10-67	Common in alkaline freshwater to brackish waters. Also reported from waters of circumneutral pH. NOTE: It was previously classified under the genus *Navicula.*
Semiorbis		Found in cold, low-conductivity, acid, mountain waters including bogs (Cox 1996, Kociolek and Spaulding 2003b). NOTE: Only one species has ever been described, *Semiorbis hemicyclus* (Ehrenb.) (Patrick 1963).
Skeletonema	10-9	Present in eutrophic waters of moderate to high conductivity depending on the species. *Skeletonema* is found in slow-moving rivers and appears to be intolerant of heavy metals and industrial pollution (Cox 1996, Palmer 1962).
Stauroneis	10-64	Benthic and planktonic in freshwater lakes and streams. Some species can be pollution tolerant.
Staurosirella	10-22	Found in fresh, usually alkaline waters attached (periphytic) to tychoplankton. It does not tolerate organic pollution. NOTE: Species of *Fragilaria lapponica, F. pinnata,* and *F. leptostauron* were separated into this genus by Williams and Round (1987). Species of *Fragilaria construens* and *F. elliptica* were separated into the genus *Staurosira* in Williams and Round (1987). They differ from *Staurosirella* by having coarser striae and being elliptical to almost round in shape.
Stenopterobia	10-38	Considered acid indicators, they are restricted to highly acidic habitats and are benthic in waters of low conductivity. This genus can be found in areas of acid mine drainage near coal mining spill piles.
Stephanodiscus	10-12	Planktonic in lakes, ponds, streams, and rivers. Most species are solitary. Colonial species are united by delicate organic threads from the edge of the valve. NOTE: This is one of the most diverse and widespread genus of freshwater diatoms in the world. This genus is an underclassified group, and one frequently finds descriptions of new species.
Surirella	10-39	Widely distributed, epipelic, benthic diatom genus found in waters with wide-ranging conductivity (freshwater to marine).

Table continued next page

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Table 10-1 Summary of ecological information for freshwater diatom genera (continued)

Genus	Figure	Habitat, Distribution, and Comments
Synedra/ Synedrella/ Ulnaria	10-23	Found in fresh, shallow water attached by jelly pores as periphyton. Some long and fine species are planktonic. NOTE: New taxonomic works lump many *Synedra* species in *Fragilaria.* In old literature, *Synedra* species are solitary, and *Fragilaria* species form chains. *Synedra* is listed as both taste and odor causing and filter clogging by APHA et al. 2005.
Tabellaria	10-16	Common in circumneutral freshwaters, but they find optimum conditions in acidic waters. Thus *Tabellaria* species can be included as acid-water indicators when in high proportion. Live cells are joined in long zigzag colonies connected by mucilaginous pads secreted through pores. They can also use the pads to attach to substrates. Many species are planktonic or can also be attached. *Tabellaria* is listed as both taste and odor causing and filter clogging by APHA et al. 2005.
Tetracyclus	10-15	Aerophilic, found on damp rocks, soil, moist walls of waterfalls, and can be found in the acid water systems (Cox 1996). Found primarily in the holarctic latitudes. Live cells form zigzag colonies linked at the end of the valve face.
Thalassiosira	10-13	Invasive in freshwater habitats. This genus is typical of high salinity and is most common in rivers and polluted ponds; salinification is associated with human disturbance.
Tryblionella	10-41	Epipelic in waters of high conductivity, either freshwater to marine. NOTE: Old literature has it as *Nitzschia.*
Urosolenia		Usually found in freshwater lakes, planktonic.

Treatment Plant

Certain diatom genera can clog filters and screens in the water treatment plants. The genera *Cyclotella, Fragilaria, Cymbella, Navicula, Synedra, Tabellaria, Melosira, Asterionella,* and *Diatoma* are listed in APHA et al. (2005) and Palmer (1962, 1977) as filter clogging. Most diatom blooms can clog filters, particularly if they are of species that produce thick mucilage. Periphytic diatom species can form thick biofilms in any surface exposed to light. The genera *Gomphonema, Encyonema, Cocconeis,* and *Achnanthidium* are of particular concern in biofilms.

Water Supply

Taste and odor. Many diatoms produce taste and odor compounds that can affect the quality of the water supply. *Asterionella, Synedra,* and *Tabellaria* are listed in APHA et al. (2005) and Palmer (1962, 1977) as "taste and odor" species. Diatoms in water supplies do not occur as pure cultures, so it is hard to say if the diatoms themselves or the bacteria they are associated with produce taste and odor compounds such as 2-methylisoborneol (MIB) or geosmin. Additional research is required on this topic.

Toxin production. Most reports of toxins produced by diatoms are related to central nervous system toxicity, induced by domoic acid produced only by a species of the brackish/marine diatom belonging to the genus *Pseudonitzschia,* which is unlikely to be found in the source of drinking water (Bates et al. 1998). Some species of *Nitzschia* have been reported to have hemolytic activity (Rangel et al. 1997). However, the main concern when finding *Nitzschia* in water supplies is the presence of organic pollutants.

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DIATOM IDENTIFICATION TO GENUS

A simple key to the most common genera of freshwater diatoms is presented. This key is based on the classification by valve ornamentation and not phylogeny. The references used for this key include Patrick and Reimer 1966, 1975; Krammer and Lange-Bertalot 1986, 1988a, 1988b; Lange-Bertalot and Moser 1994; Compère 2000; Collins and Kalinsky 1977; Hustedt 1930, 1985; Kingston 2003; Kociolek and Spaulding 2003a, 2003b; Lowe 2003; Siver et al. 2005; Van Heurck 1896; Round et al. 1990; Sheath and Wehr 2003; and others. Taxonomy is constantly in a state of flux, especially at the genus level, because large groupings are separated as scanning electron microscopy techniques are employed. This key includes the genera most likely to be found by the drinking water industry, noting when possible the most recent taxonomic designations.

KEY TO COMMON FRESHWATER GENERA

1a. Valve with radial symmetry (centric), 2

1b. Valves not radially symmetrical, 13

2a. Valves are cylindrical or barrel-shaped, often in chains, 3

2b. Valves are not cylindrical but flattened like a Petri dish, 8

3a. Valve margin with spines, 4

3b. Valve margin does not have obvious spines; margins appear smooth, 6

4a. Valves have dots or pores on the girdle face. They form chains so they are usually seen in girdle view, *Aulacoseira* (Figure 10-5)

4b. Valves without obvious dots or pores, instead feature intercalary bands that seem to overlap (see Figure 10-6), 5

5a. A single long spine present on each valve, *Urosolenia*

5b. Two elongated spines present on each valve, *Acanthoceras* (Figure 10-6)

6a. Valves have punctae ornamentation on the valve face, *Pleurosira* (Figure 10-7)

6b. Valves do not have distinct ornamentation on the valve face, 7

7a. Valve appears to have thickened walls, *Melosira* (Figure 10-8)

7b. Valves appear very delicate and may be difficult to see, sometimes barely distinguishable from background material, *Skeletonema* (Figure 10-9)

8a. Edge of valve margin contains spines/teeth (spine length <2× width), 10

8b. Edge of valve without visible spines or teeth; valve appears smooth, 9

9a. Margin of valve face with 2–4 ocelli that are opposite each other (at "corners" on girdle view), *Pleurosira* (Figure 10-7)

9b. Margin not as described above, with marginal ornamentation (costae); small pores may be present in the central area, *Cyclotella* (Figure 10-10)

10a. Central area of valve face distinctly punctated but different than marginal area. It appears to be striated from the margin inward, *Cyclostephanos* (Figure 10-11)

10b. Central area of valve with little or no differentiation from the margin, 11

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11a. Valve edge with obvious spines; puncta paired to form what looks like spokes of a wheel, *Stephanodiscus* (Figure 10-12)

11b. Valve edge without obvious spines; may have small projections (teeth) that appear as tiny dark circles, 12

12a. Ornamentation uniform across valve face; obvious pores in central area may form geometric patterns in some species, *Coscinodiscus*
Most are now considered marine, (see *Thalassiosira*)

12b. Valve face with areolae arranged in radial tangential rows. Valve with marginal fultoportulae and may also have a few isolated fultoportulae in the central area, *Thalassiosira* (Figure 10-13)

13a. Raphe absent on both valves (Araphidae), 14

13b. Raphe present on at least one valve, 24

14a. Frustule with inner septa originating from the girdle bands (see Figure 10-14), 15

14b. Frustule without inner septa, 16

15a. Transapical costae (heavy silica deposits that appear as dark lines) present, *Tetracyclus* (Figure 10-15)

15b. No transapical costae, *Tabellaria* (Figure 10-16)

16a. Costae present in addition to striae on valve, 17

16b. Costae not present, 18

17a. Valve and/or girdle view heteropolar (one end of the valve larger than the other); girdle view wedge-shaped, *Meridion* (Figure 10-17)

17b. Valve view and/or girdle view isopolar, *Diatoma* (Figure 10-18)

18a. Valves asymmetrical to at least one axis, 19

18b. Valves symmetrical to all axes, 22

19a. Valves are strongly curved in the longitudinal axis (crescent shaped or semicircular), 20

19b. Valves straight, 21

20a. Valve has ventral swelling in central area, *Hannaea* (Figure 10-19)

20b. Valve without swelling; central area continuous/entire (very rare), *Semiorbis*

21a. Valves elongated with capitate (swollen and rounded) ends; heteropolar, *Asterionella* (Figure 10-20)

21b. Valve without capitate ends; has parallel striae throughout valve, *Martyana* (Figure 10-21)

22a. Valves broadly elliptical or star-shaped (cruciform), *Staurosirella* (Figure 10-22)

22b. Valve appears elongate and not as above, 23

23a. Valves usually needle or javelin shaped, *Synedra/Synedrella/Ulnaria* (Figure 10-23)

NOTE: *Fragilaria, Staurosirella, Fragilariforma, Synedra, Synedrella, Ulnaria,* and other related genera under the old classification were all grouped into

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Synedra or *Fragilaria*. They were distinguished as *Synedra* when found isolated or solitary, and as *Fragilaria* when found in colonial formations. Taxonomy of this group is often being revised, and species are being separated into new genera.

23b. Valve not as above, *Fragilaria, Fragilariforma* (Figure 10-24)

24a. Raphe present on only one valve (Monoraphidae), 25

24b. Raphe present on both valves, 31

25a. Frustule distinctly oval or wedge-shaped, 26

25b. Frustule not oval; can be elongated, elliptical, or linear, 28

26a. In girdle view, cuneate (bent wedge or bent cone); septa present, *Rhoicosphenia* (Figure 10-25)

NOTE: Actually biraphid, but raphe on one valve is rudimentary and very difficult to observe.

26b. No septa present, not wedge-shaped in girdle view, but can be curved in girdle view (most often observed in colonies), 27

27a. Valve oval-shaped to almost circular, punctated radial striae on both valves; rapheless valve convex, and raphe valve often has striae interrupted at the margins by a clear area, *Cocconeis* (Figure 10-26)

27b. Striae rarely visible, not radial throughout valve; small frustule (<20 μm) with colonies in girdle view showing bent or slightly curved valves, *Achnanthidium* (some species = *A. minutissima*) (Figure 10-27)

28a. Striae appear thickened and rapheless valve has a horseshoe-shaped structure in central area, *Planothidium* (Figure 10-28)

28b. Striae not thickened and without "horseshoe" area, 29

29a. Central striae tightly clustered to appear as a depression in center of valve, *Achnanthidium* (Figure 10-27)

29b. Striae not as described above, 30

30a. Parallel striae throughout, bluntly lanceolate valve, *Rossithidium* (Figure 10-29)

30b. Striae not parallel throughout valve, *Achnanthes* (Figure 10-30)

NOTE: Species in this genus are now mostly marine.

31a. Complete raphe on both valves (Biraphidae), 33

31b. Incomplete raphe (restricted to poles) on both valves (Brachyraphidae), 32

32a. Valve heteropolar, *Actinella* (Figure 10-31)

32b. Valve isopolar, *Eunotia* (Figure 10-32)

33a. Keel (thickened silica ridge extending longitudinally along valve) present, 34

33b. Keel not present, 44

34a. Central (medial) raised keel, 35

34b. Keel in another position than described above, 36

NOTE: *Nitzschia dissipata* can be problematic because it has a slightly off-center keel raphe.

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35a. Fibulae (thick keel puncta) clearly present, *Bacillaria* (Figure 10-33)

35b. Fibulae absent, *Plagiotropis* (Figure 10-34)

36a. Keel is marginal and raised into winglike projections, 37

36b. No winglike projections, 41

37a. Frustule is saddle-shaped and very large, *Campylodiscus* (Figure 10-35)

37b. Frustule is not saddle-shaped, 38

38a. Undulations (waves) along apical axis of valve, *Cymatopleura* (Figure 10-36)

38b. Undulations absent, 39

39a. Frustule is twisted in girdle view, *Entomoneis* (Figure 10-37)

39b. Frustule not twisted, 40

40a. Valve can be isopolar or sigmoid ("S" shaped), narrow and elongated (>8× longer than wide); canal raphe is marginal and striae are fine, *Stenopterobia* (Figure 10-38)

40b. Valves generally wider (1–6× longer than wide), sometimes heteropolar, *Surirella* (Figure 10-39)

41a. Thickened costae (dark transverse structures in light microscopy) present, *Denticula* (Figure 10-40)

41b. No costae present, 42

42a. Undulation across transapical axis, *Tryblionella* (Figure 10-41)

42b. Undulation absent, 43

43a. Fibulae (keel puncta) present on the same side of both valves, *Hantzschia* (Figure 10-42)

43b. Keel puncta on opposite sides of both valves, *Nitzschia* (Figure 10-43)

44a. Costae present on valve, 45

44b. Costae absent, 46

45a. Raphe V-shaped (two arcs coming together at the center of the valve), *Epithemia* (Figure 10-44)

45b. Raphe not as described above, *Rhopalodia* (Figure 10-45)

46a. Valve shape or ornamentation symmetrical to both apical and transapical axis (see Figure 10-46), 57

46b. Valve or ornamentation asymmetrical to either apical or transapical axes (see Figure 10-47), 47

47a. Valve heteropolar (asymmetrical to transapical axis) (see Figure 10-47), 48

47b. Valve isopolar (symmetrical to transapical axis) (see Figure 10-47), 52

48a. Frustule sigmoid ("S" shaped), 49

48b. Frustule not sigmoid, 50

49a. Transapical striae and longitudinal striae perpendicular to each other, *Gyrosigma* (Figure 10-48)

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49b. Transapical and longitudinal striae not perpendicular (oblique) to each other, *Pleurosigma* (Figure 10-49)

50a. Isolated puncta present at the central area, 51

50b. Isolated puncta absent, *Rhoicosphenia* (Figure 10-25)

NOTE: Not actually under Biraphidae, but if only one valve [raphe valve] is present, the key will lead one to this point.

51a. Valve contains longitudinal shadow lines crossing striae; striae are double punctated, *Gomphoneis* (Figure 10-50)

51b. No longitudinal shadow lines crossing striae, *Gomphonema* (Figure 10-51a) or *Didymosphenia* (Figure 10-51b)

52a. Valve asymmetrical to apical axis, 53

(Refer to illustration at key number 10-46b [Figure 10-47].)

52b. Valve symmetrical to apical axis, 56

(Refer to illustration at key number 10-46a [Figure 10-46].)

53a. Valve has swollen central area and straight raphe, *Reimeria* (Figure 10-52)

53b. Valve without swollen central area, 54

54a. Biconvex raphe of both valves seen at the same time; girdle bands present; hyaline area may also be present above raphe center (proximal fissures), *Amphora* (Figure 10-53)

54b. Raphe not as described above, 55

55a. Distal (outer) ends of raphe deflected dorsally ("smiles"); with or without stigmata (elongated pore) located on the ventral side of the raphe, *Cymbella* (Figure 10-54)

55b. Distal ends of raphe deflected ventrally ("frowns"); stigmata usually absent or if present is found on the dorsal side of the raphe, *Encyonema* (Figure 10-55)

56a. Isolated stigmata (elongated pore) at central area, *Luticola* (Figure 10-56)

56b. No isolated stigmata (elongated pore) at central area, *Anomoeoneis* (Figure 10-57)

57a. Girdle band has internal chambers (may look like teeth in girdle view) called partecta or locules, *Mastogloia* (Figure 10-58)

57b. No partecta present, 58

58a. Thickened band or clearing surrounding the raphe, 59

58b. No thickened band around raphe, 61

59a. Coarse, robust striae; band appears as canal; shape elliptical, *Diploneis* (Figure 10-59)

59b. Not as described above, 60

60a. Distal ends of raphe have pencil point ends and thickened band extends from poles toward center of valve, *Frustulia* (Figure 10-60)

60b. Distal ends of raphe without pencil point ends; and clearing limited to poles, *Amphipleura* (Figure 10-61)

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61a. Valve edges with long marginal lines formed by inner canals; circular central hyaline area; central raphe ending usually curved in opposite directions, *Neidium* (Figure 10-62)

61b. Valve not as described above, 62

62a. Hyaline area with thickened silica across transapical axes at central raphe ends (forming a cross or X-shape), 63

62b. Valve not described as above, 64

63a. Characteristic "X" at central nodule of valve, *Capartogramma* (Figure 10-63)

63b. Clear band of silica perpendicular to raphe forming a cross ("+") at central nodule of valve, *Stauroneis* (Figure 10-64)

64a. Striae appear solid and unobstructed; may have large hyaline area around raphe, parallel or perpendicular to raphe, 65

64b. Striae not as described above, 66

65a. Shadow lines cross striae near valve margin (edge), *Caloneis* (Figure 10-65)

65b. No shadow lines present, *Pinnularia* (Figure 10-66)

66a. Valve with thickened bluntly rounded ends that make poles appear clear of striae, *Sellaphora* (Figure 10-67)

66b. Valve poles otherwise, 67

67a. Parallel striae down the entire length of valve, *Craticula* (Figure 10-68)

67b. Striae otherwise, 68

68a. Irregularly spaced puncta create longitudinal lines parallel to raphe; may have small, diamond-shaped central area, *Brachysira* (Figure 10-69)

68b. Puncta not as described above, 69

69a. Small oblong cell with depression at central hyaline area; short striae usually not visible; raphe appears as a thin line, *Diadesmis* (Figure 10-70)

69b. Valve not as described above, *Navicula* and related genera (Figure 10-71)

NOTE: Some species of *Navicula* have been placed into separate genera. For simplicity's sake, these genera—*Placoneis*, *Cavinula*, *Geissleria*, *Hippodonta,* and others—have been kept in the *Navicula* group.

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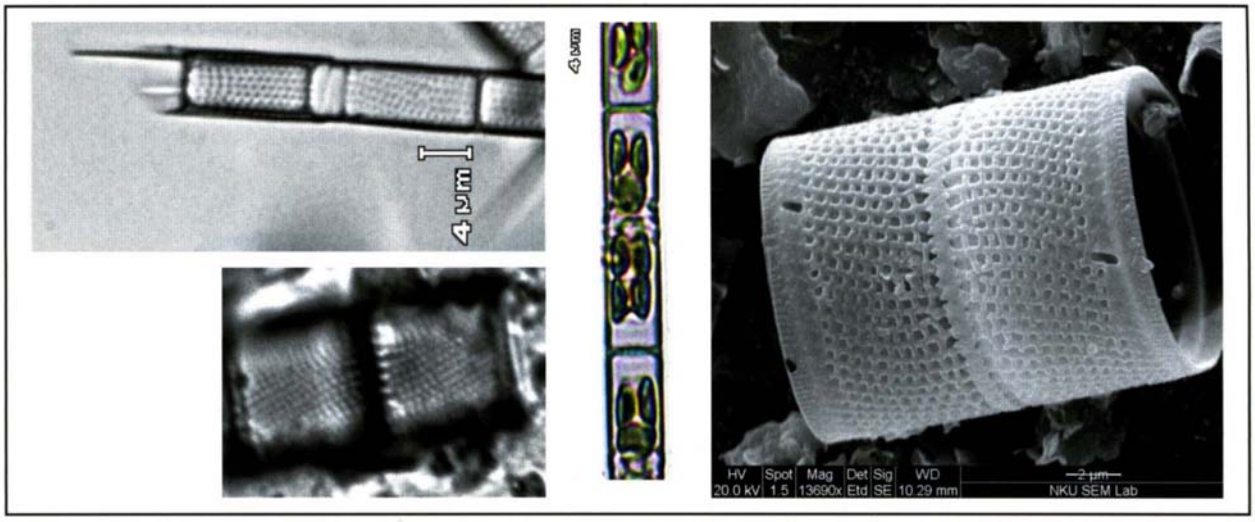

Figure 10-5 *Aulacoseira*

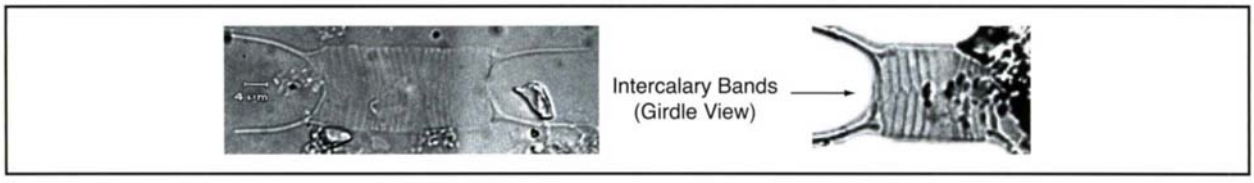

Figure 10-6 *Acanthoceras*

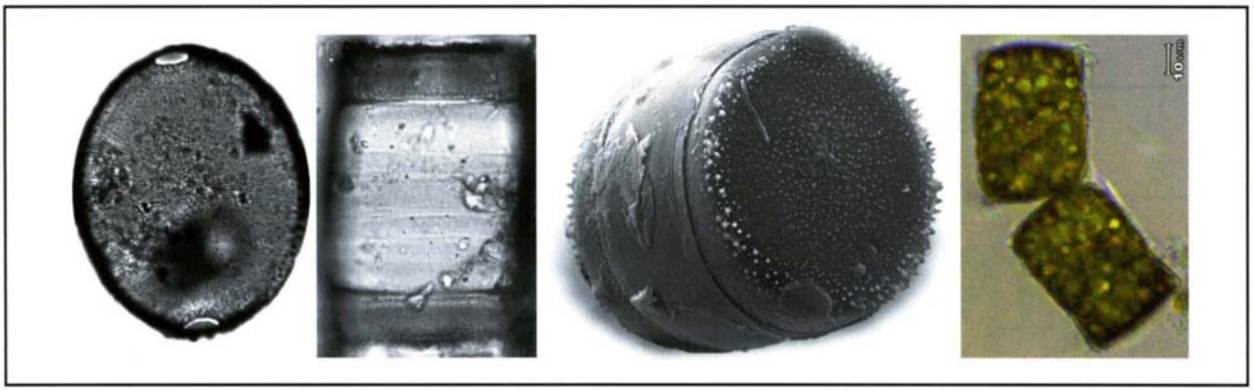

Figure 10-7 *Pleurosira*

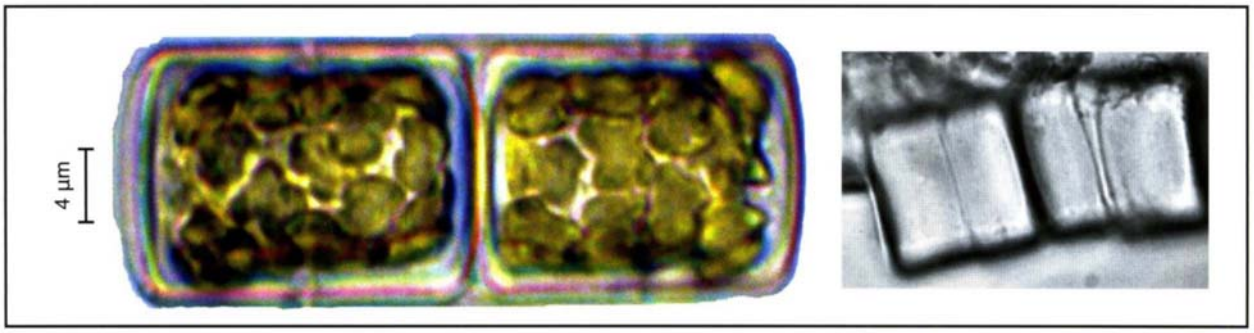

Figure 10-8 *Melosira*

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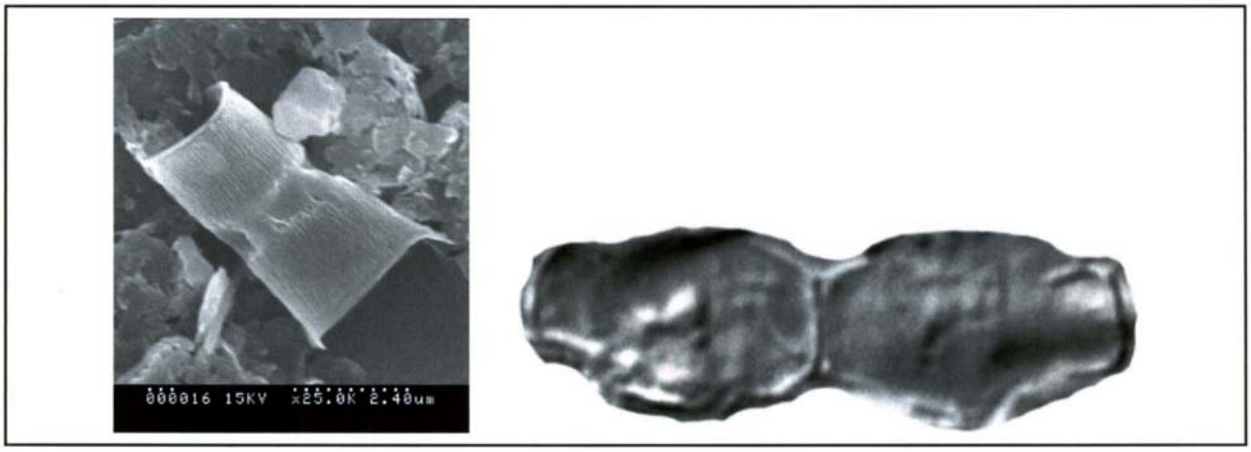

Figure 10-9 *Skeletonema*

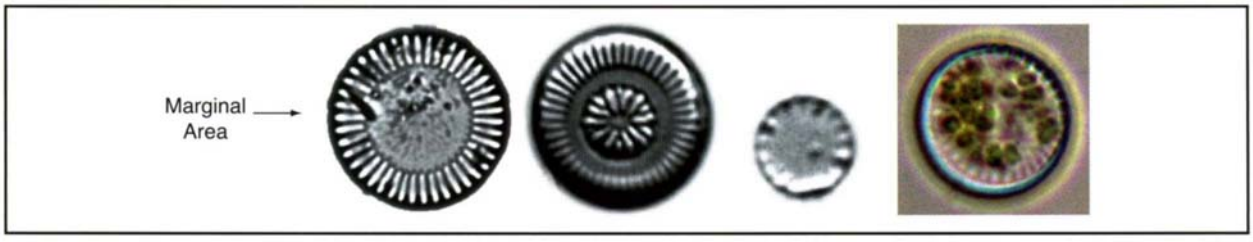

Figure 10-10 *Cyclotella*

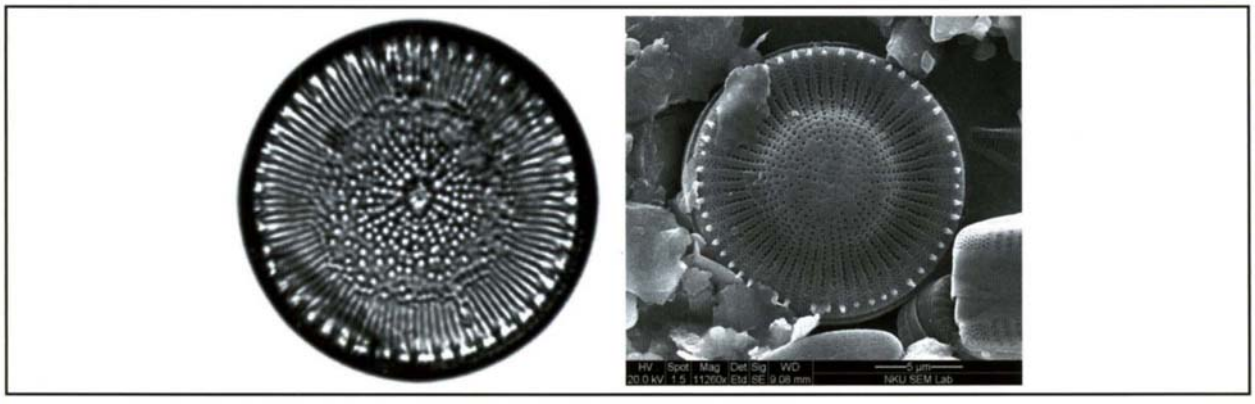

Figure 10-11 *Cyclostephanos*

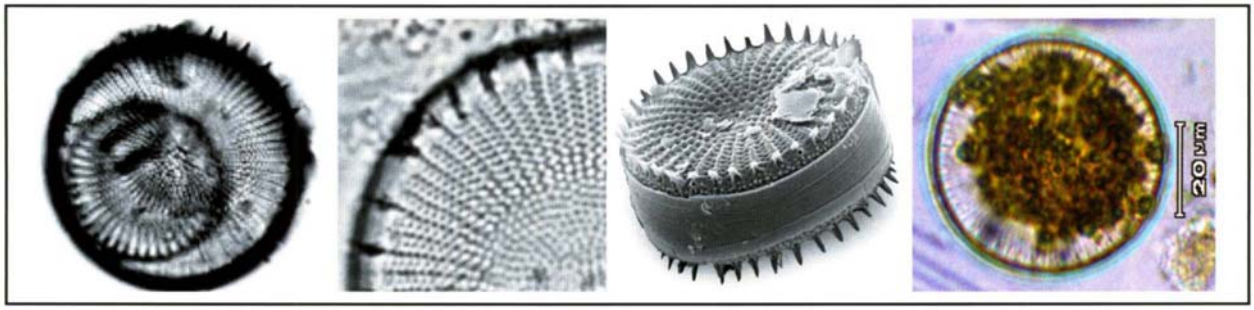

Figure 10-12 *Stephanodiscus*

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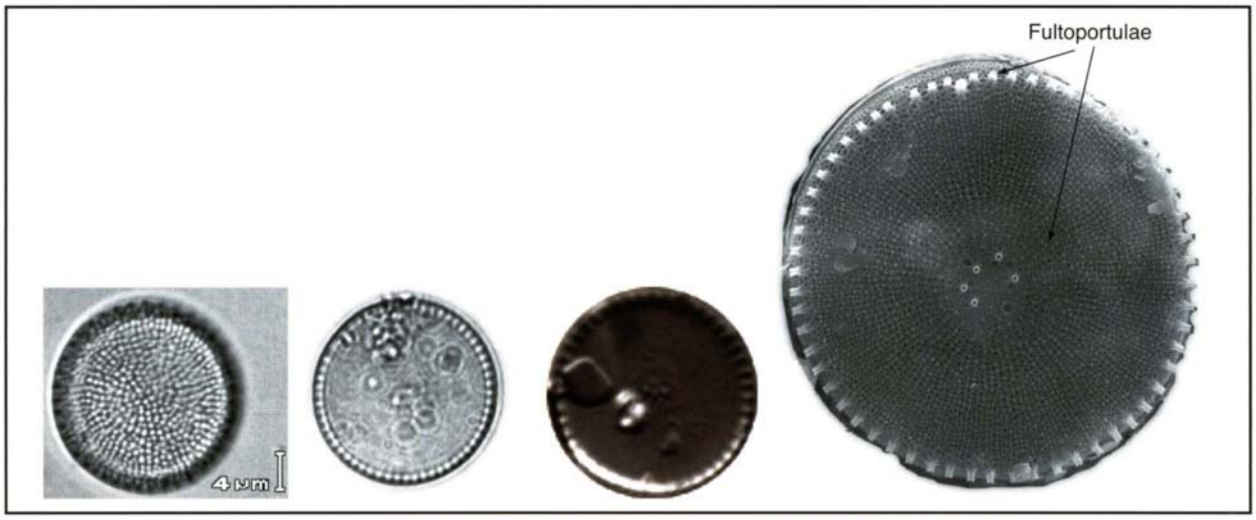

Figure 10-13 *Thalassiosira*

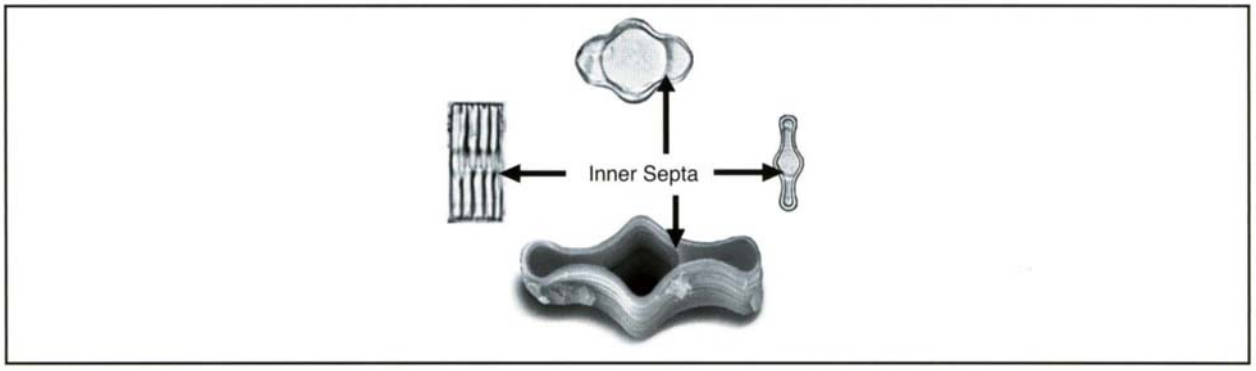

Figure 10-14 Septa

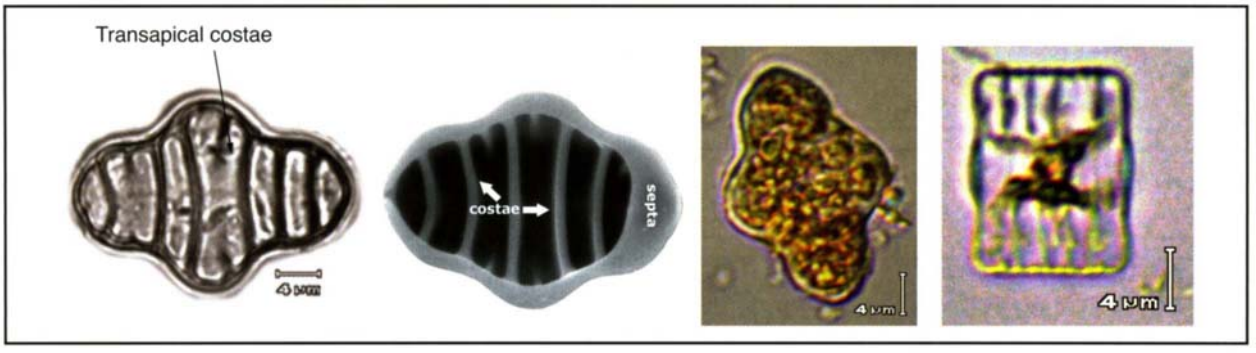

Figure 10-15 *Tetracyclus*

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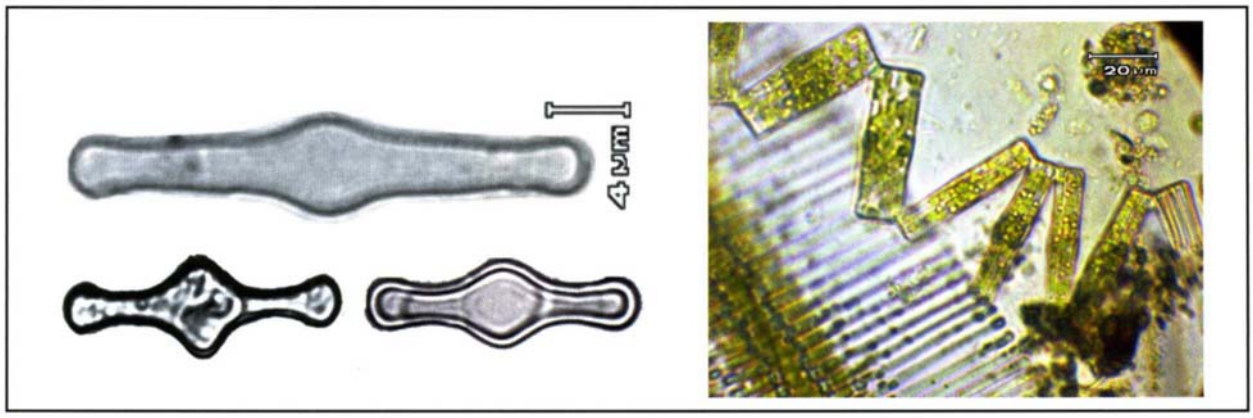

Figure 10-16 *Tabellaria*

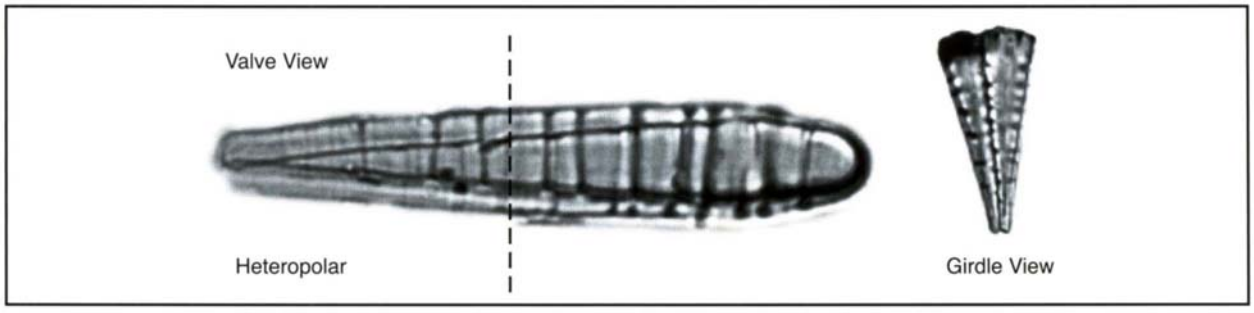

Figure 10-17 *Meridion*

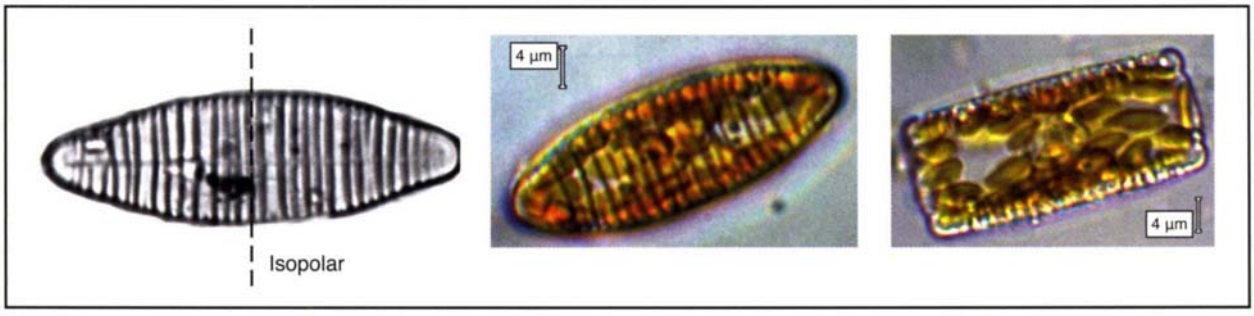

Figure 10-18 *Diatoma*

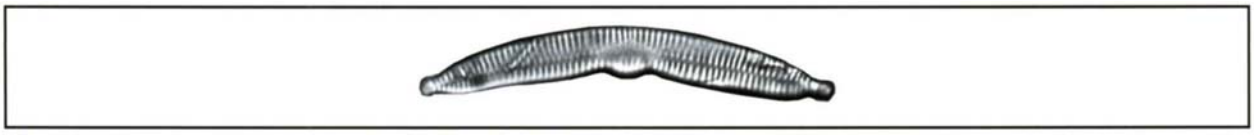

Figure 10-19 *Hannaea*

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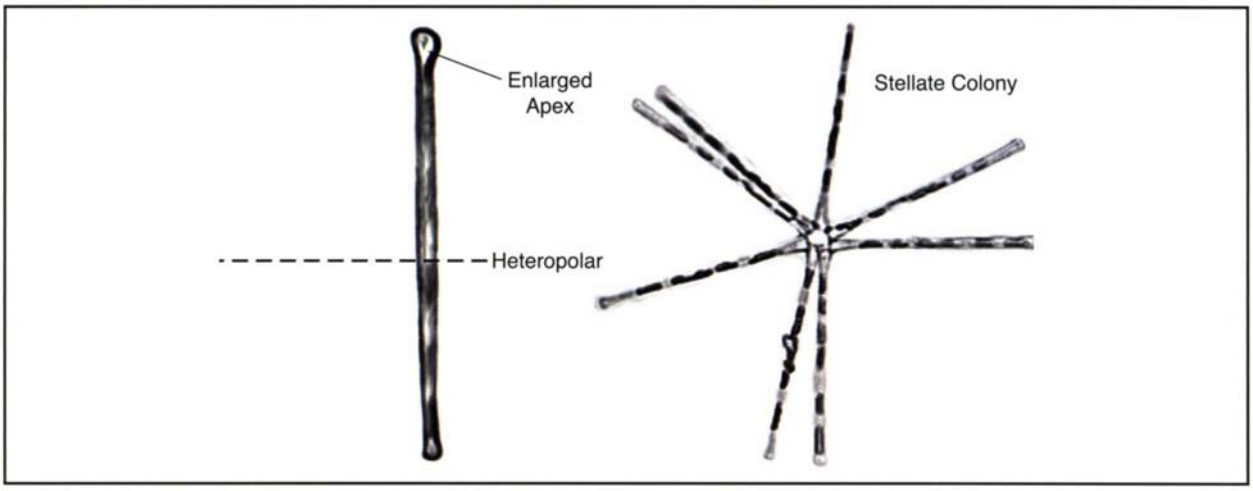

Figure 10-20 *Asterionella*

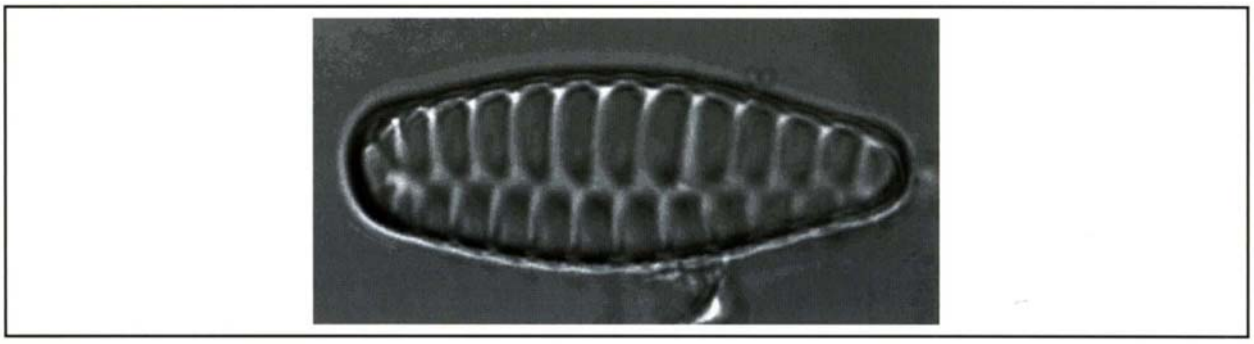

Figure 10-21 *Martyana*

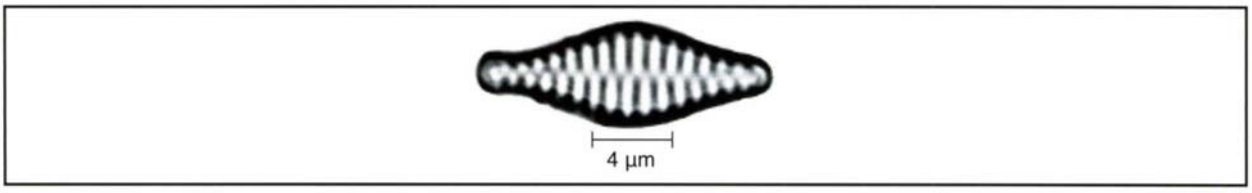

Figure 10-22 *Staurosirella*

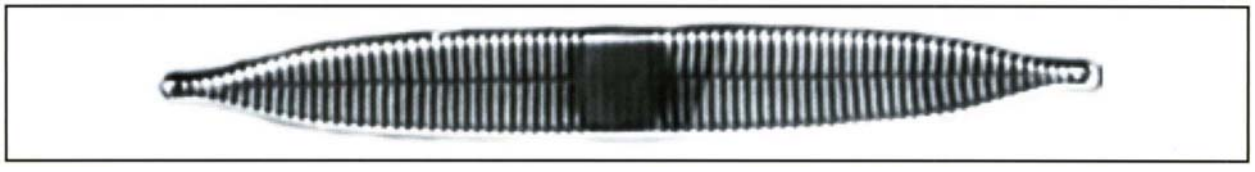

Figure 10-23 *Synedra/Synedrella/Ulnaria*

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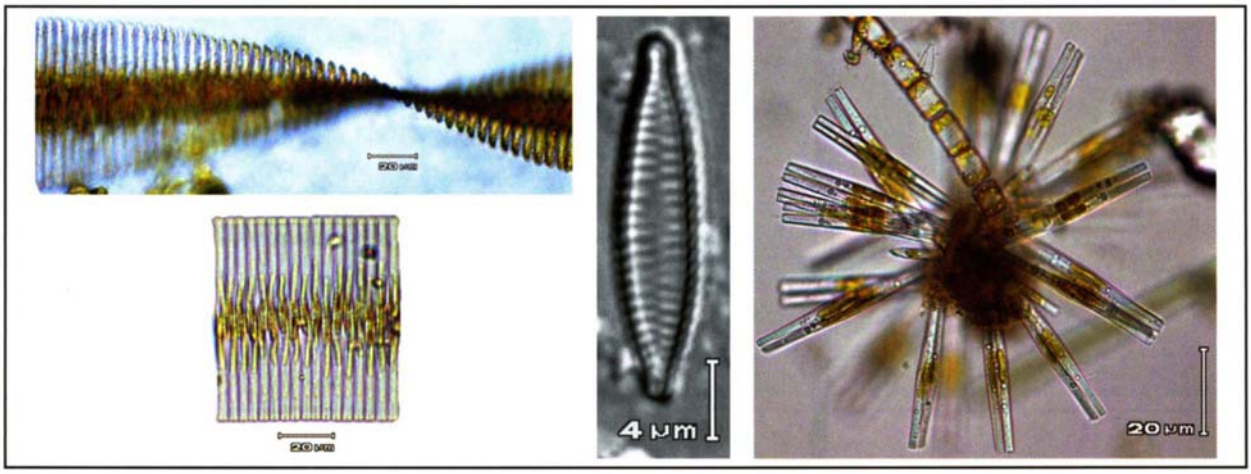

Figure 10-24 *Fragilaria* and related genera

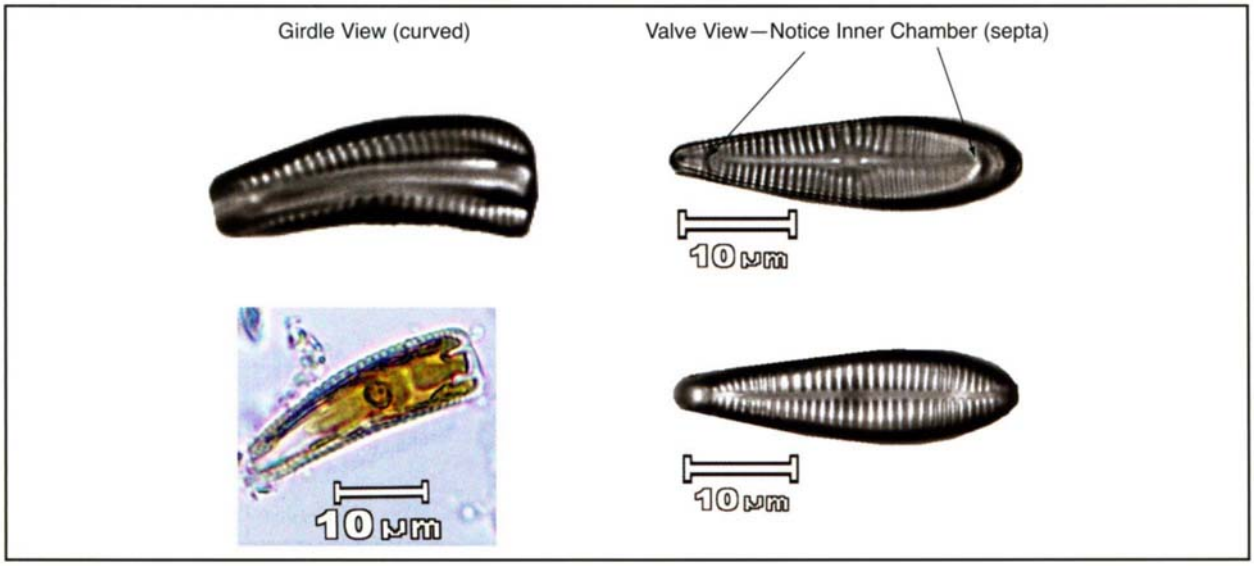

Figure 10-25 *Rhoicosphenia*

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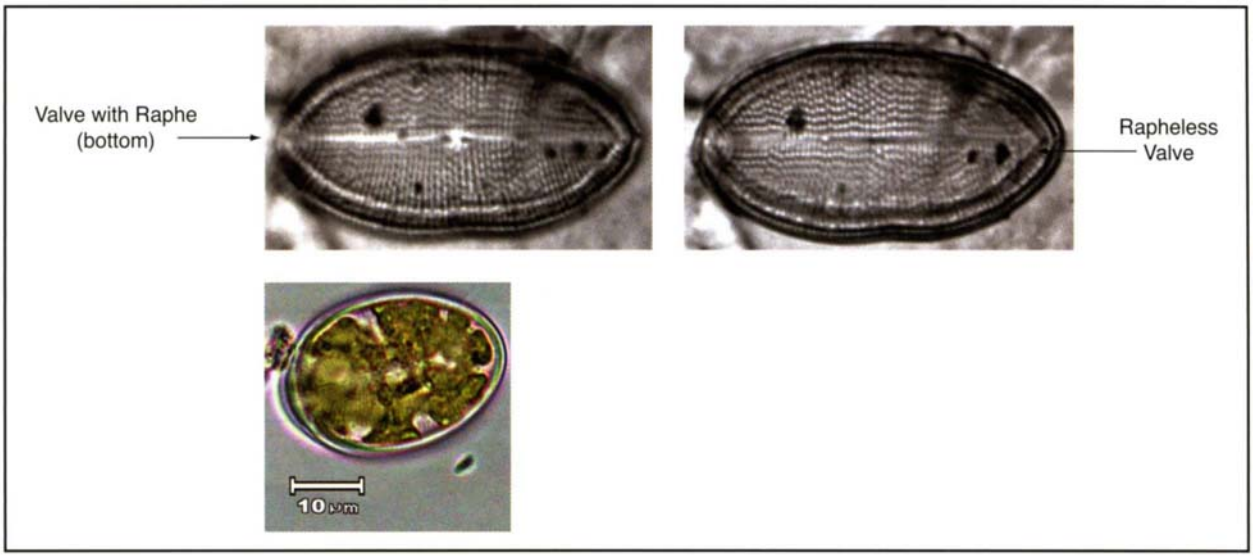

Figure 10-26 *Cocconeis*

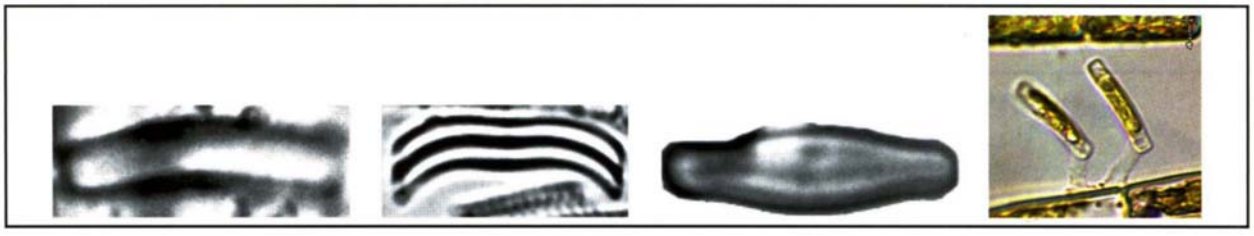

Figure 10-27 *Achnanthidium*

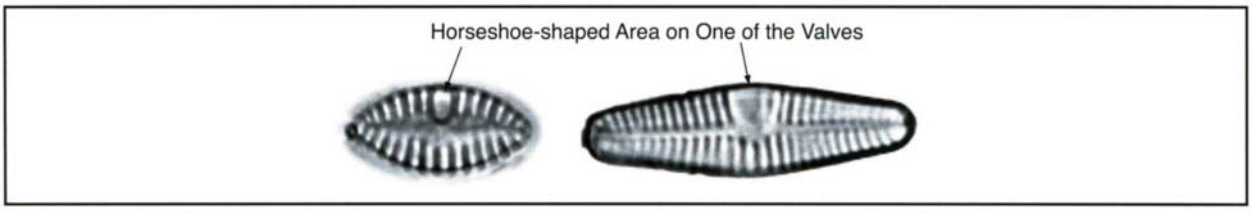

Figure 10-28 *Planothidium*

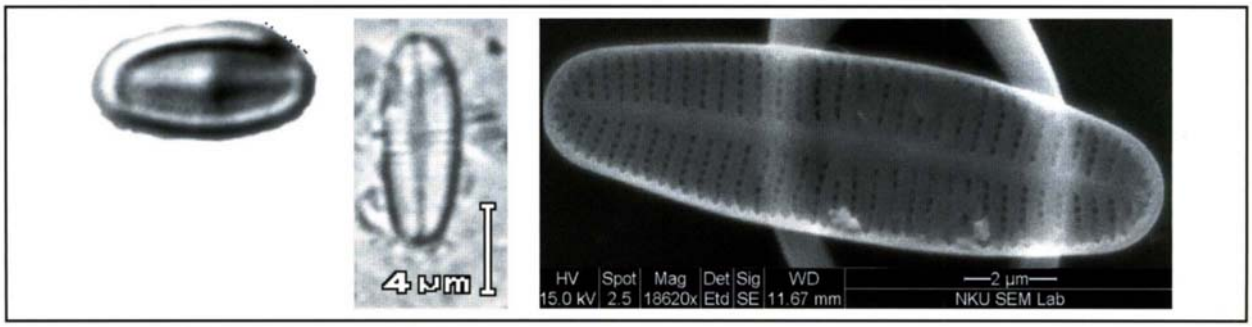

Figure 10-29 *Rossithidium*

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Figure 10-30 *Achnanthes*

Figure 10-31 *Actinella*

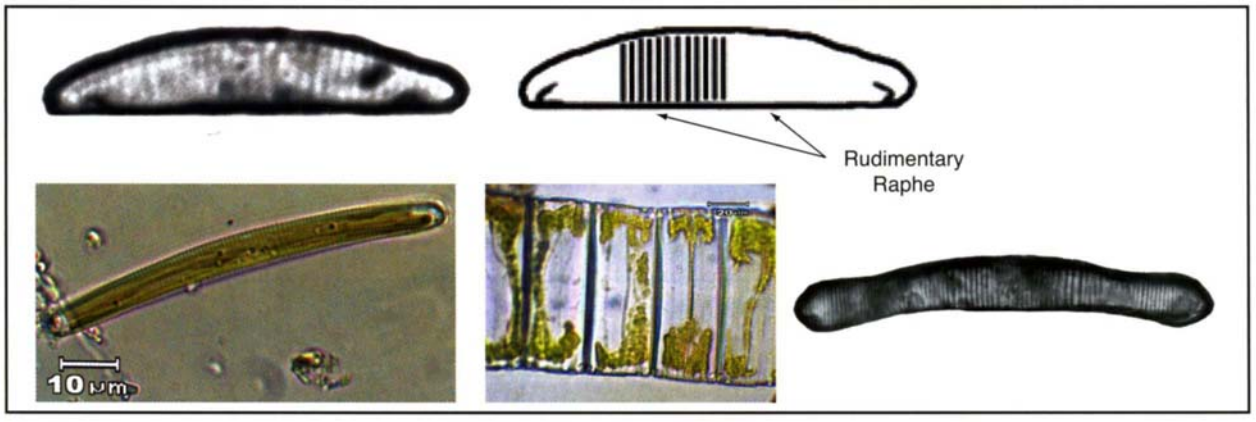

Figure 10-32 *Eunotia*

Figure 10-33 *Bacillaria*

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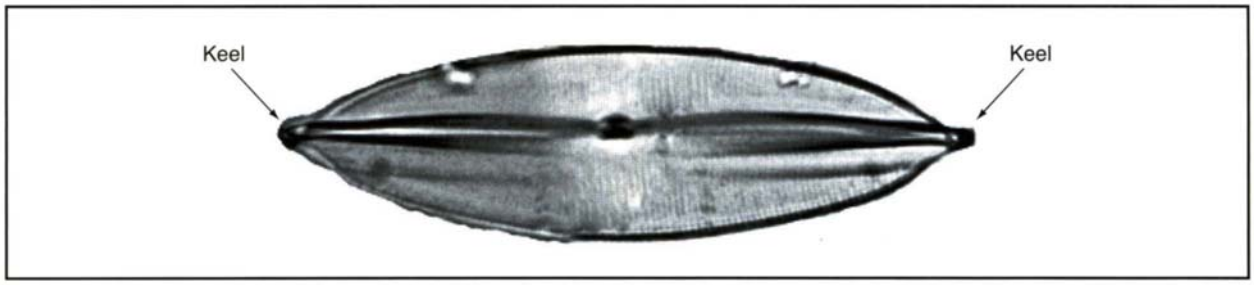

Figure 10-34 *Plagiotropis*

Figure 10-35 *Campylodiscus*

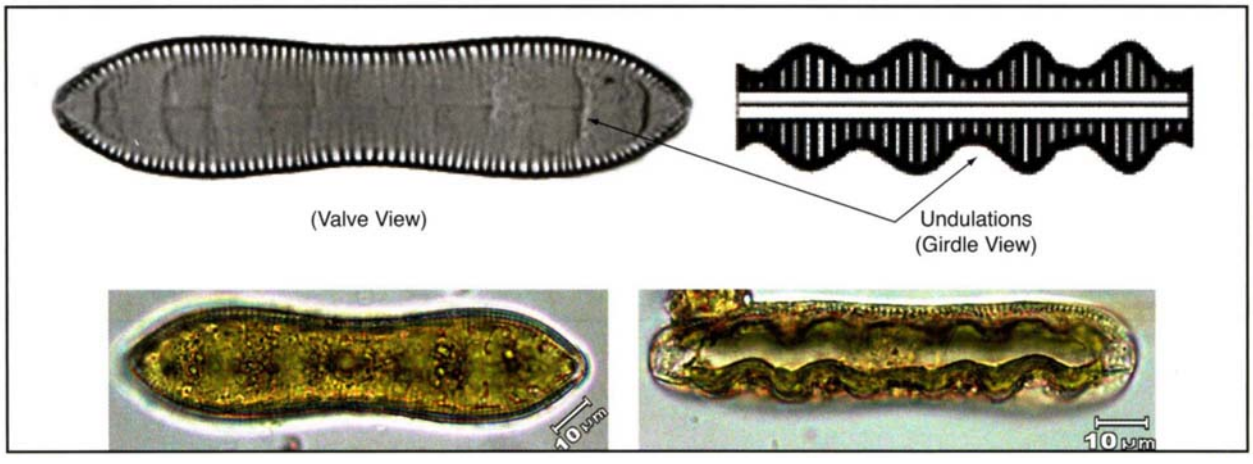

Figure 10-36 *Cymatopleura*

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Figure 10-37 *Entomoneis*

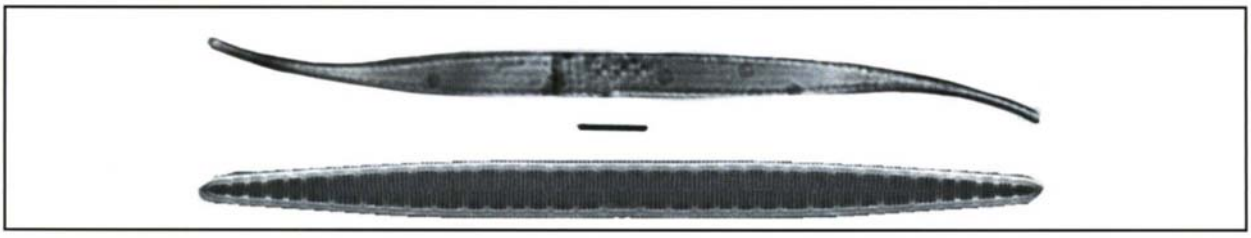

Figure 10-38 *Stenopterobia*

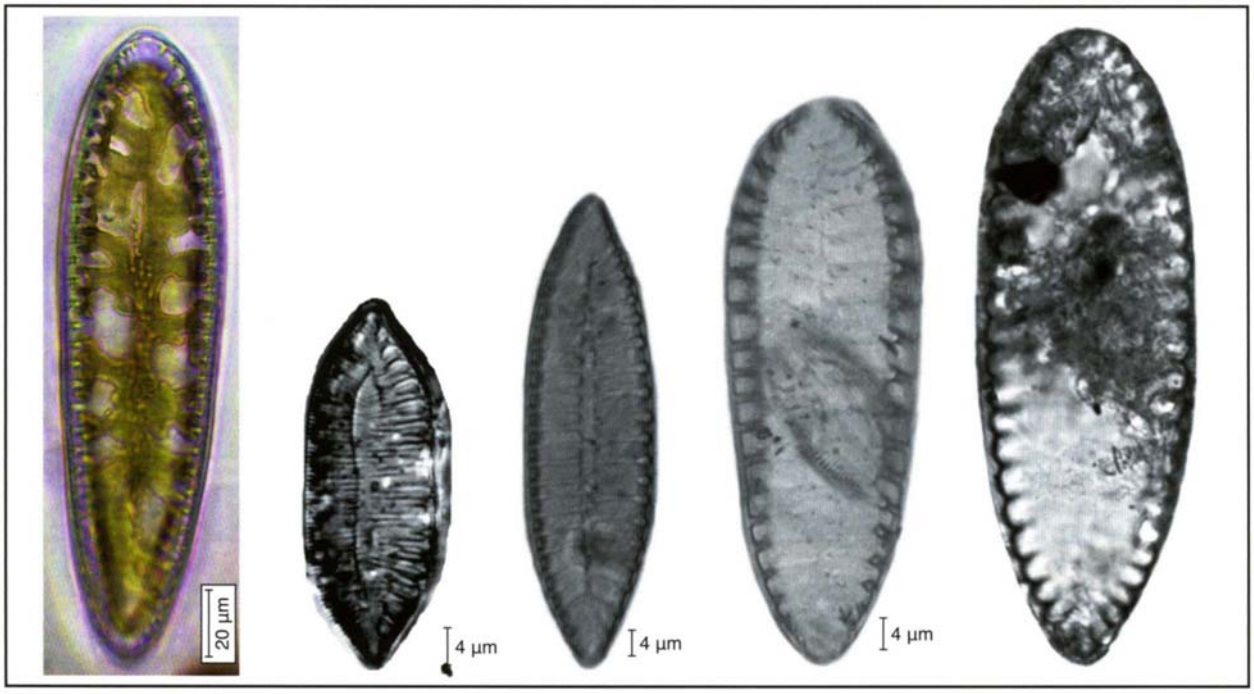

Figure 10-39 *Surirella*

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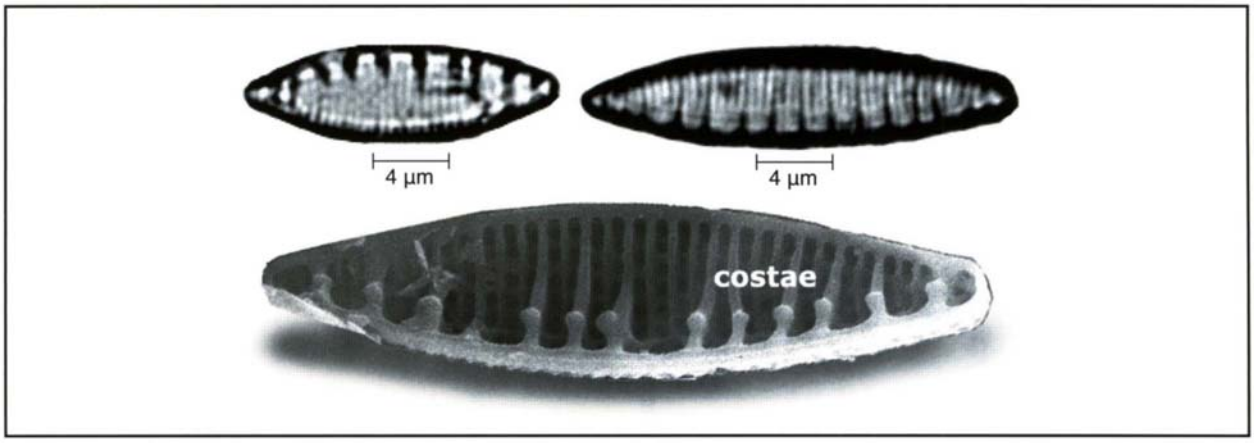

Figure 10-40 *Denticula*

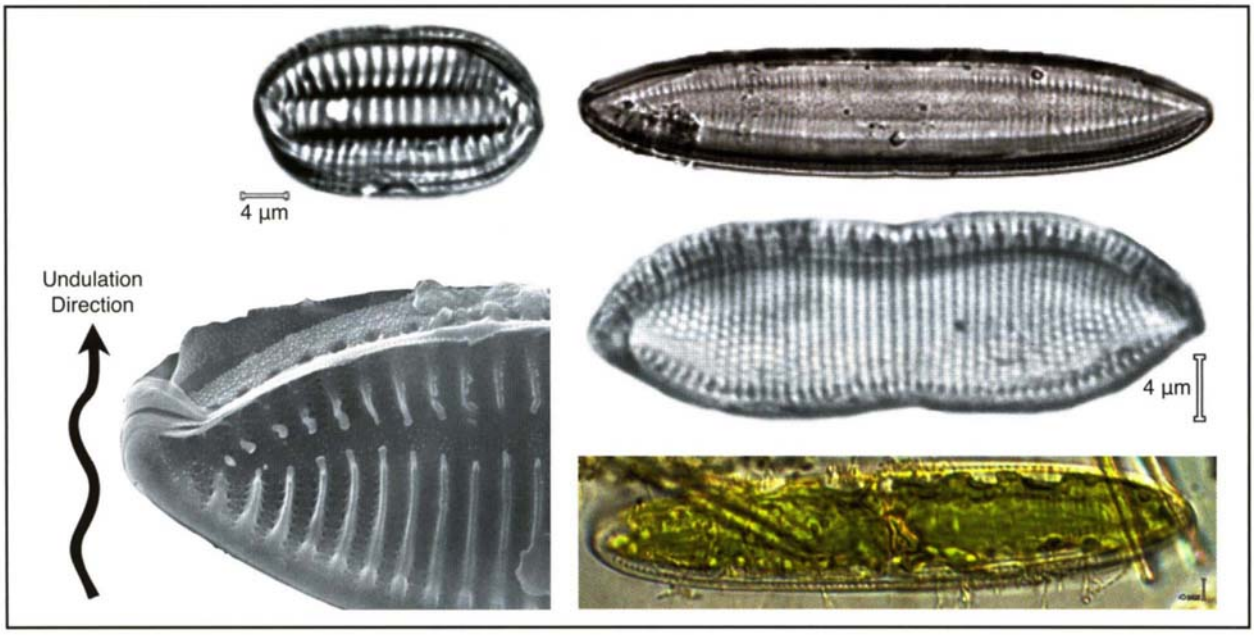

Figure 10-41 *Tryblionella*

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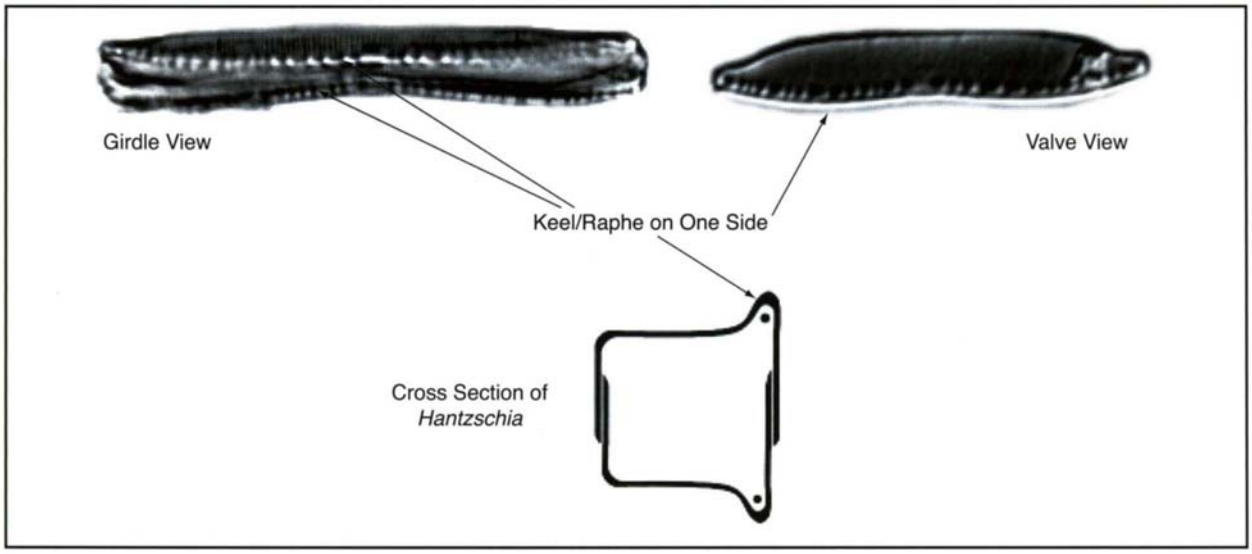

Figure 10-42 *Hantzschia*

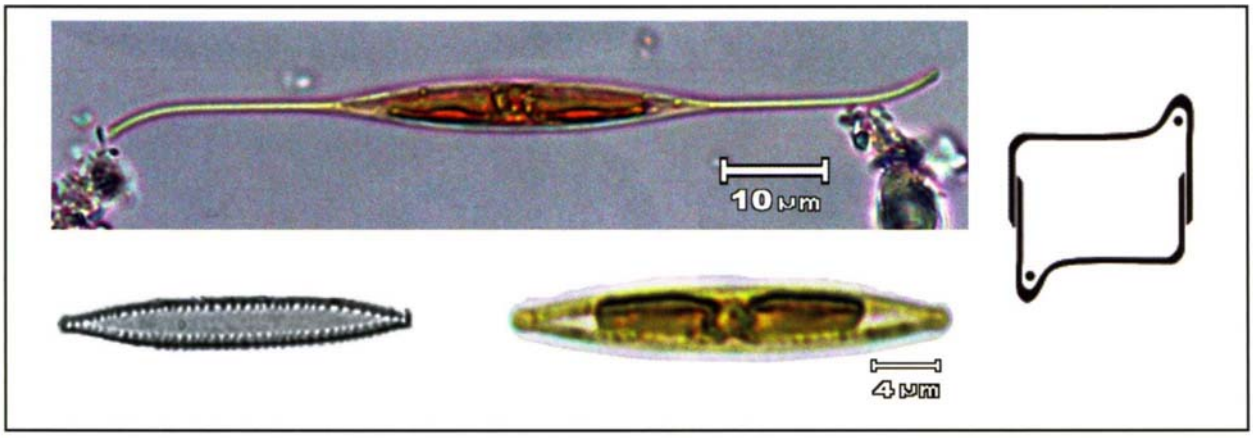

Figure 10-43 *Nitzschia*

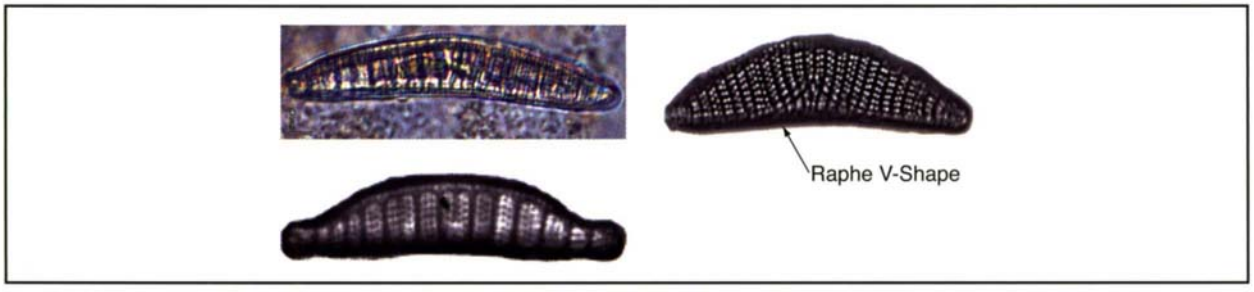

Figure 10-44 *Epithemia*

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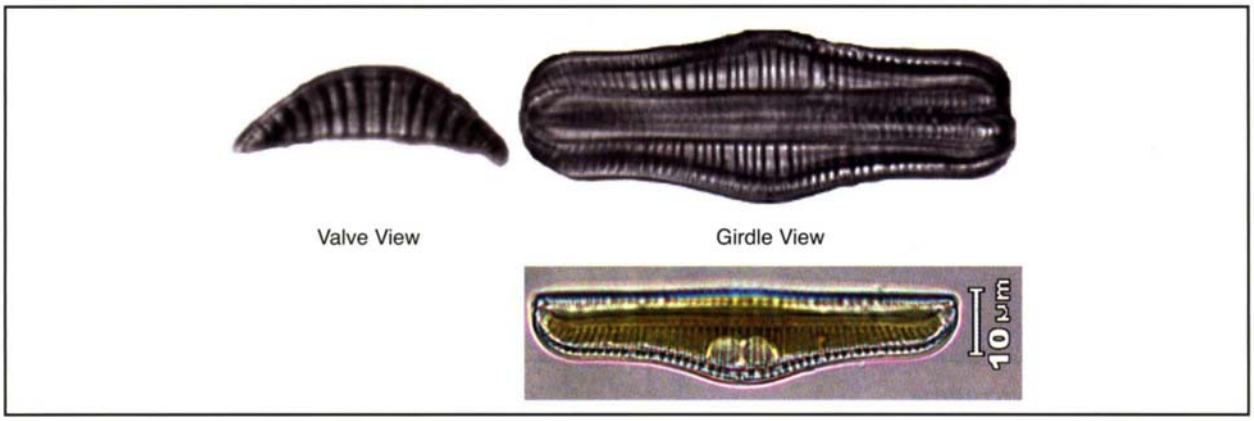

Figure 10-45 *Rhopalodia*

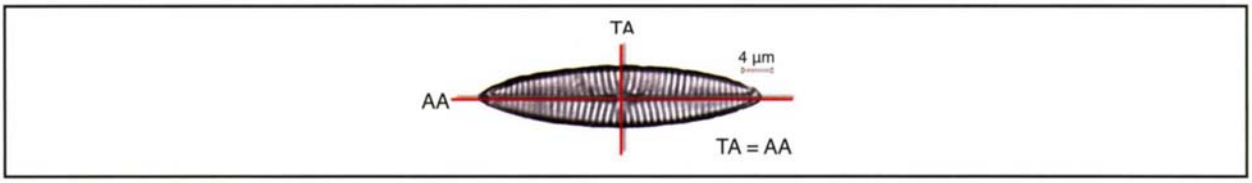

Figure 10-46 Symmetrical axis of symmetry in diatoms (AA = apical axis, TA = transapical axis)

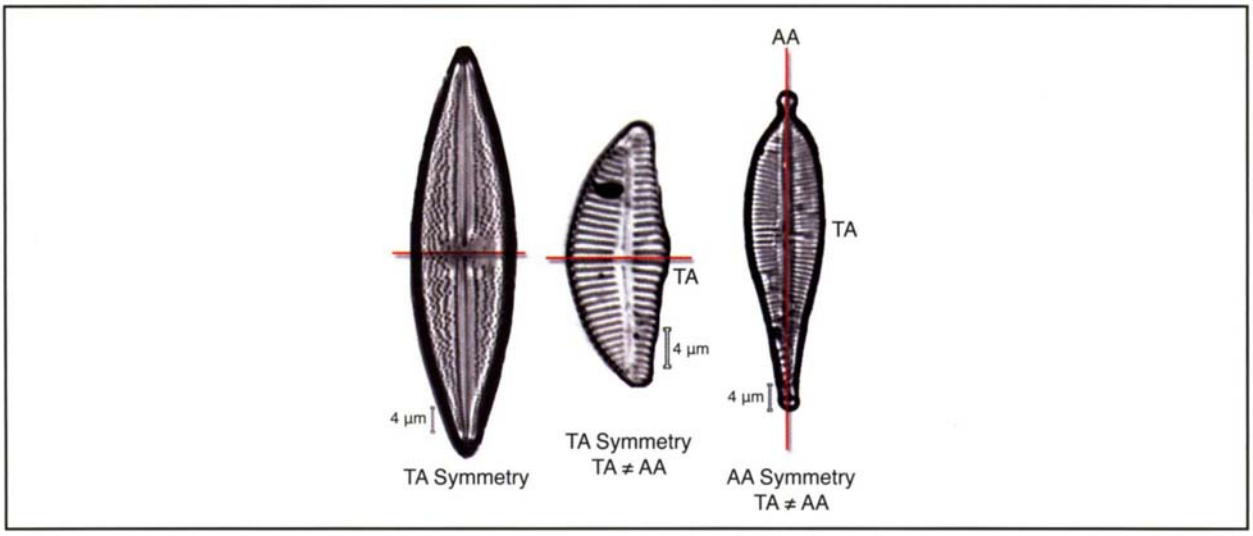

Figure 10-47 Asymmetrical axis of symmetry in diatoms (AA = apical axis, TA = transapical axis)

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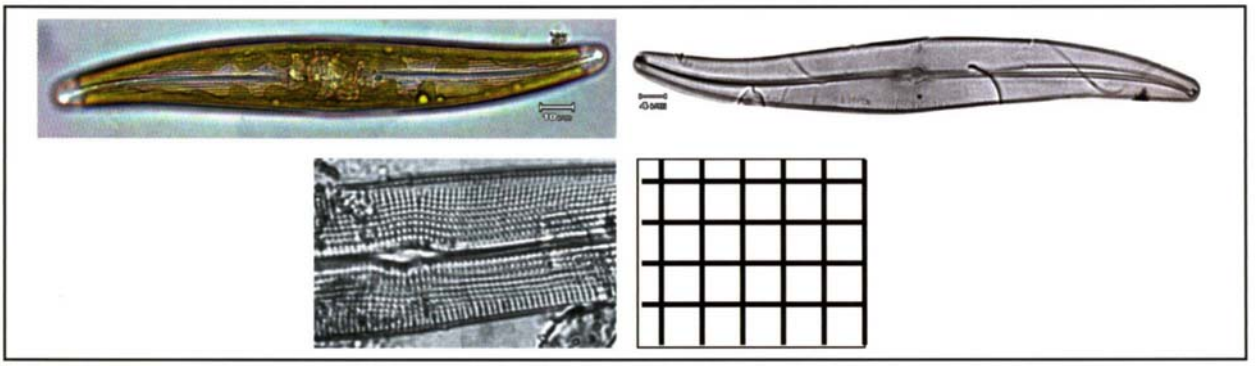

Figure 10-48 *Gyrosigma*

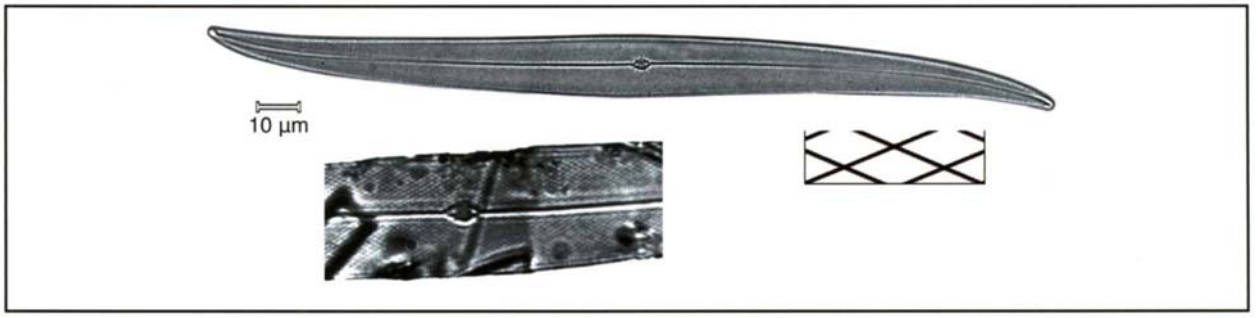

Figure 10-49 *Pleurosigma*

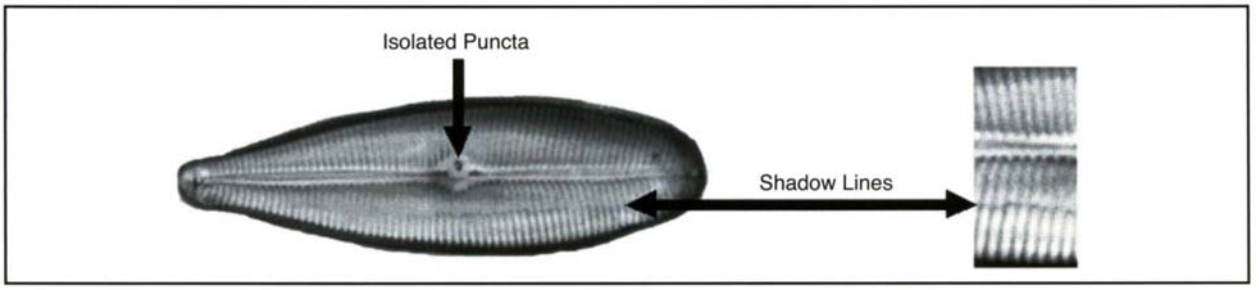

Figure 10-50 *Gomphoneis*

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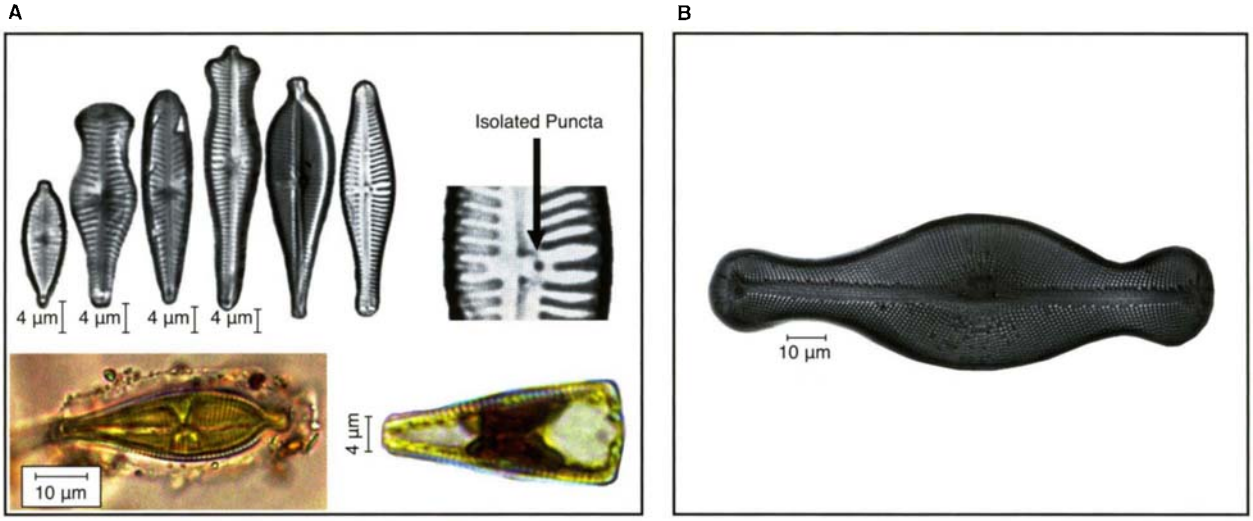

Figure 10-51 (A) *Gomphonema*; (B) *Didymosphenia geminata*

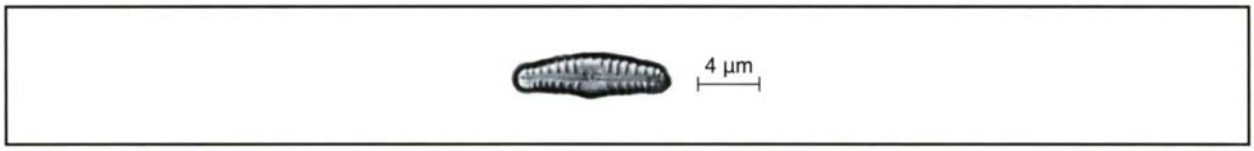

Figure 10-52 *Reimeria*

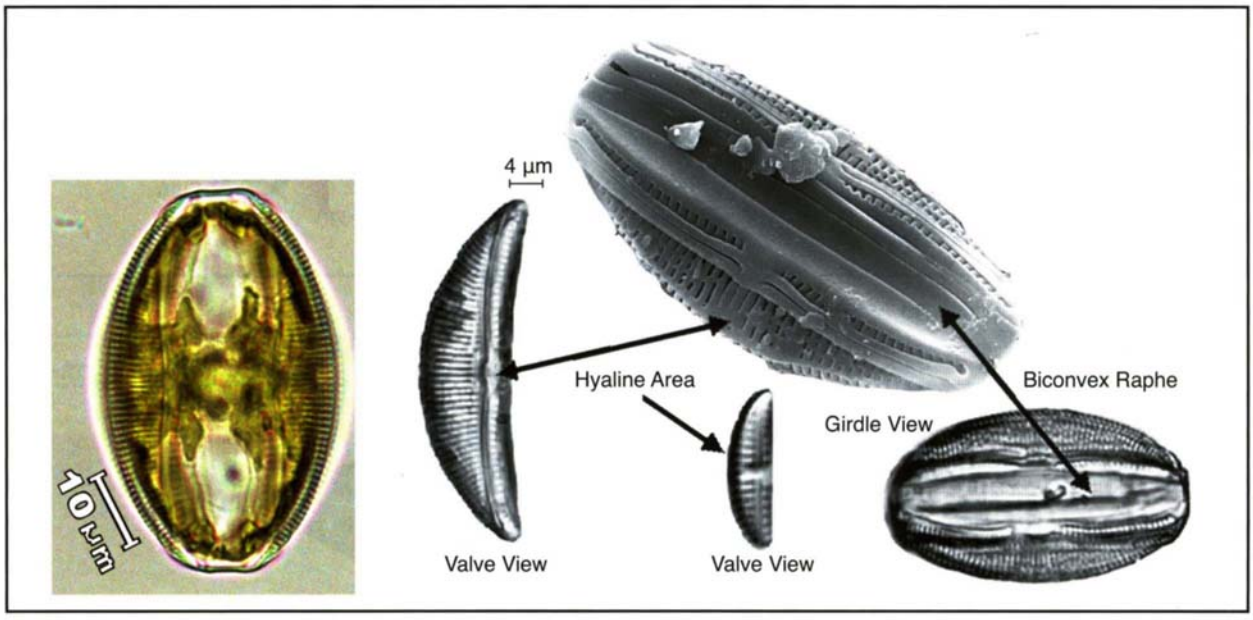

Figure 10-53 *Amphora*

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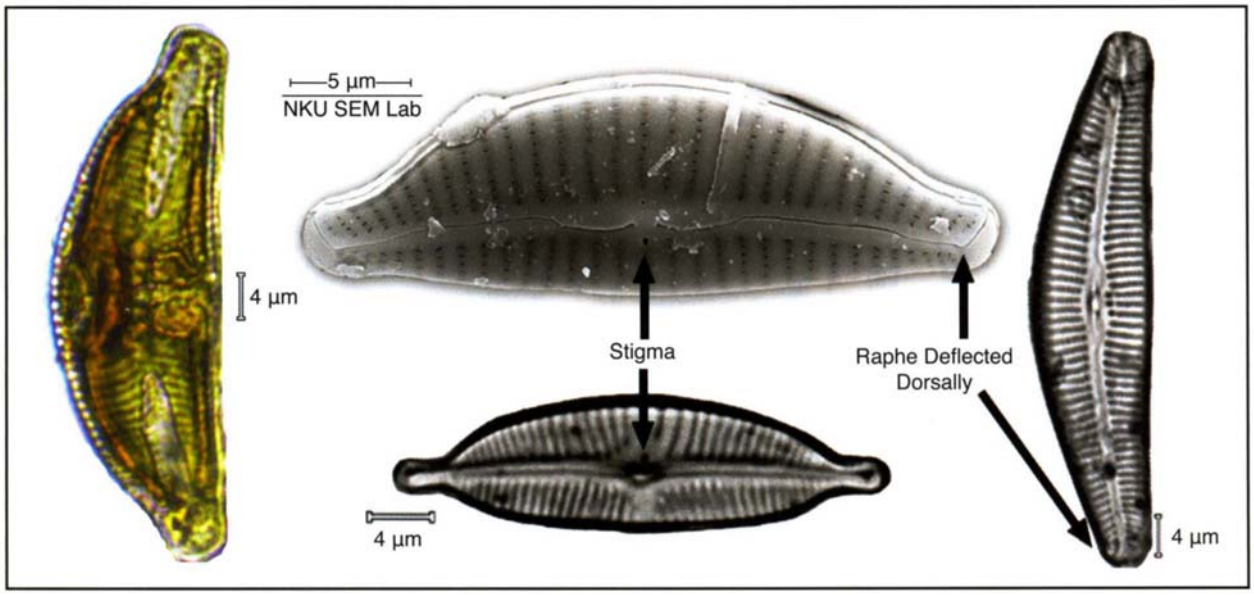

Figure 10-54 *Cymbella*

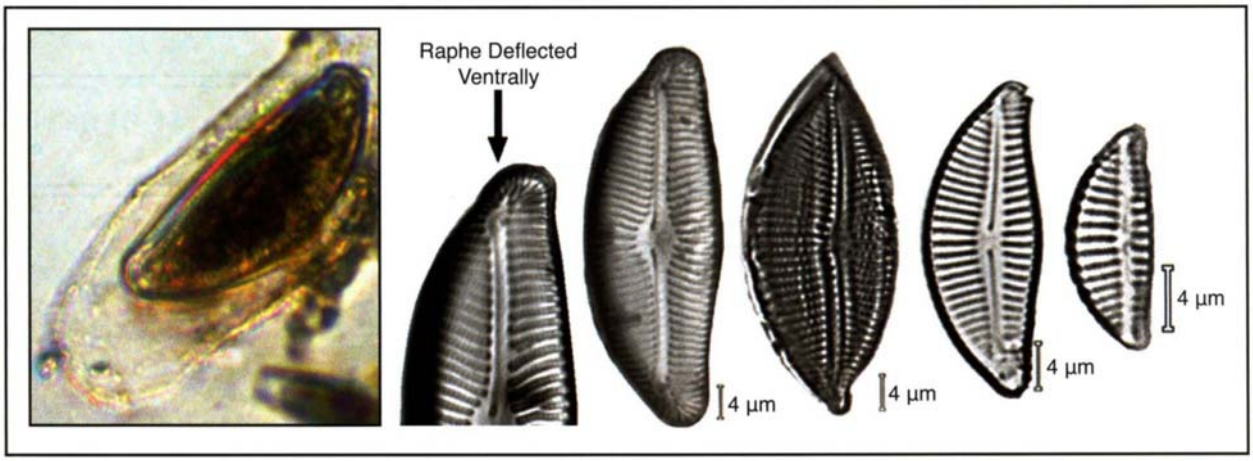

Figure 10-55 *Encyonema*

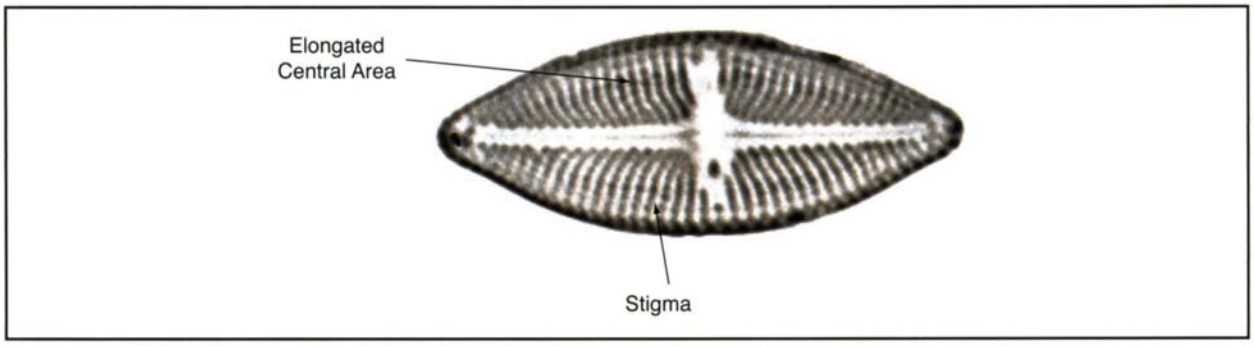

Figure 10-56 *Luticola*

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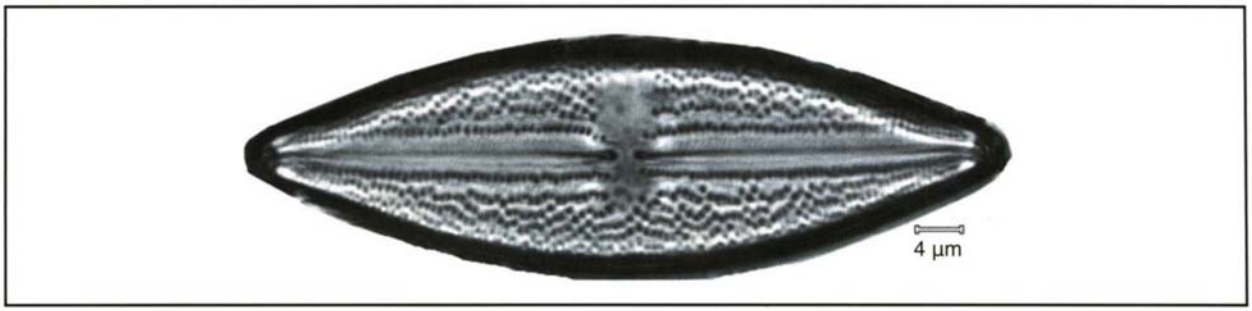

Figure 10-57 *Anomoeoneis*

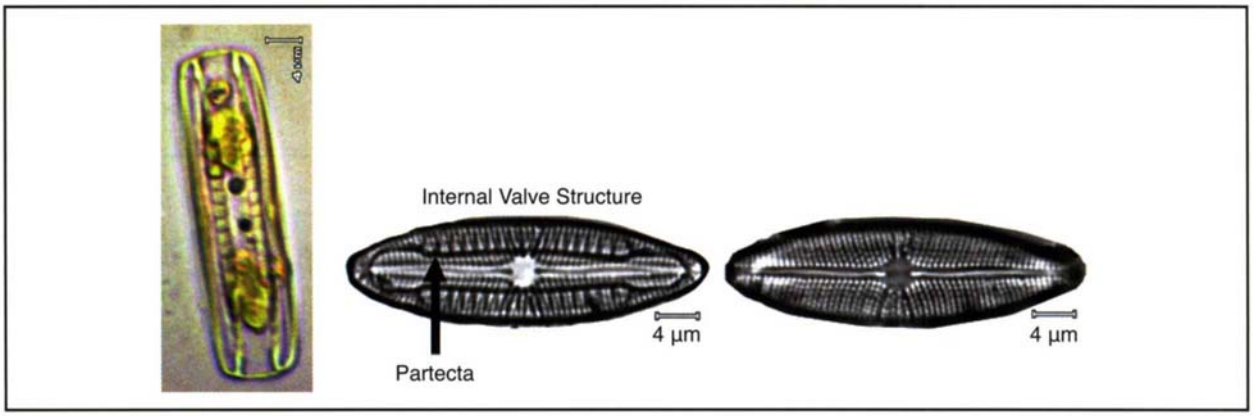

Figure 10-58 *Mastogloia*

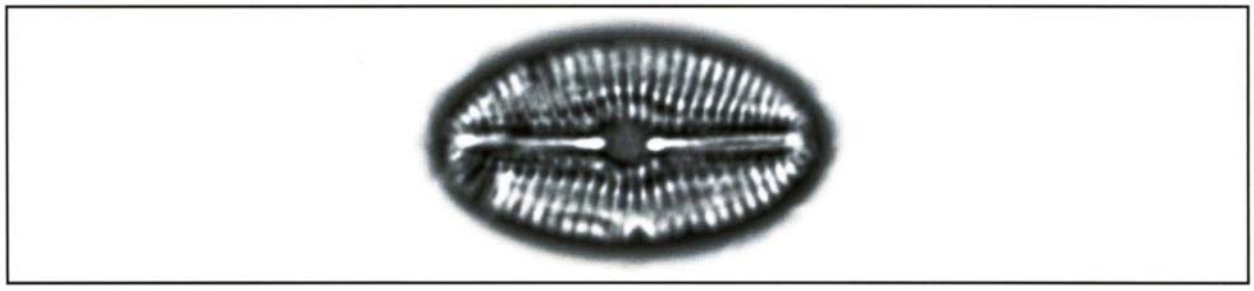

Figure 10-59 *Diploneis*

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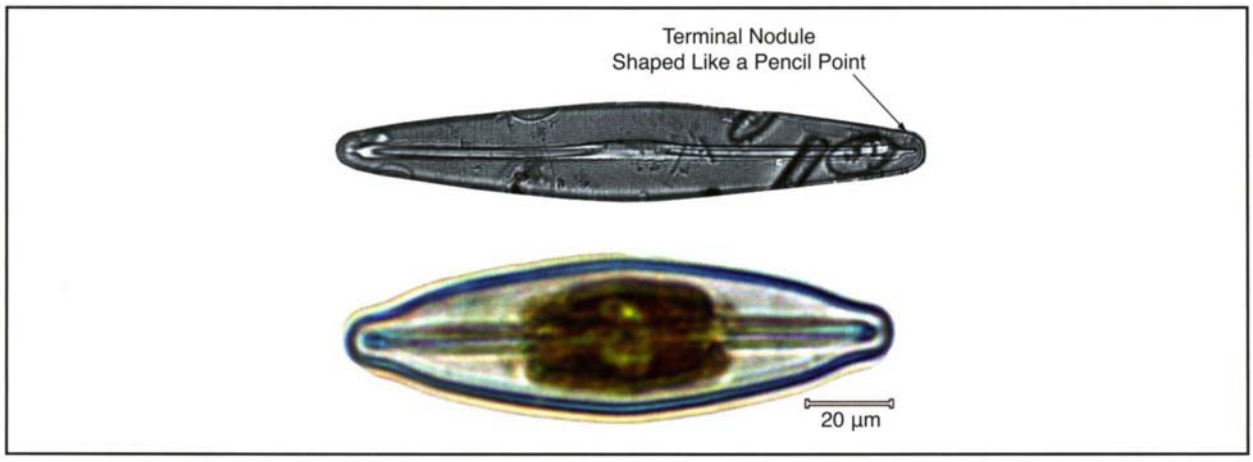

Figure 10-60 *Frustulia*

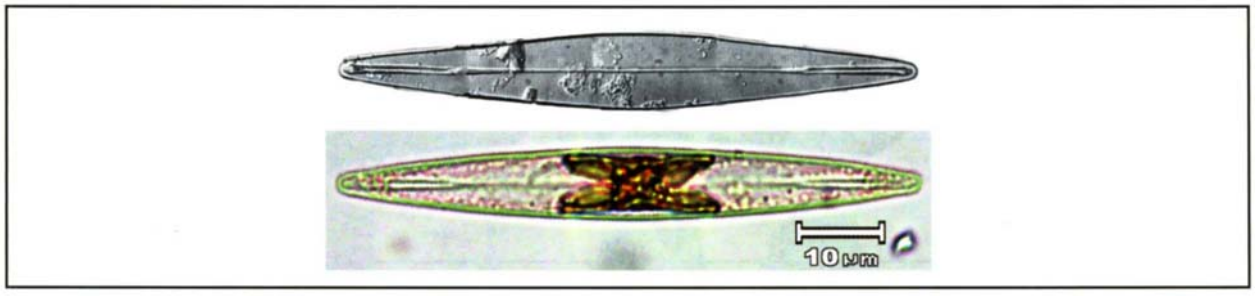

Figure 10-61 *Amphipleura*

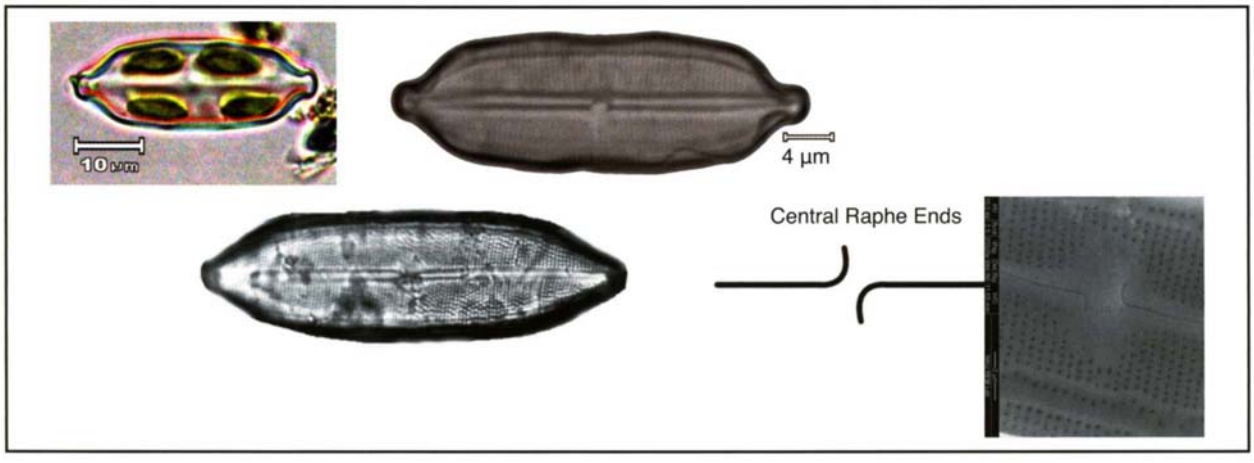

Figure 10-62 *Neidium*

Figure 10-63 *Capartogramma*

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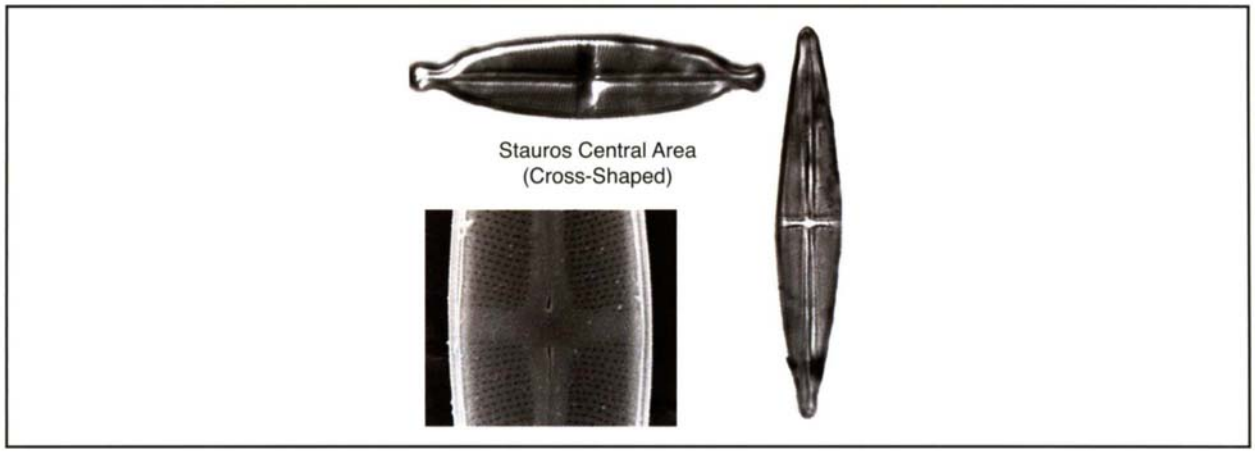

Figure 10-64 *Stauroneis*

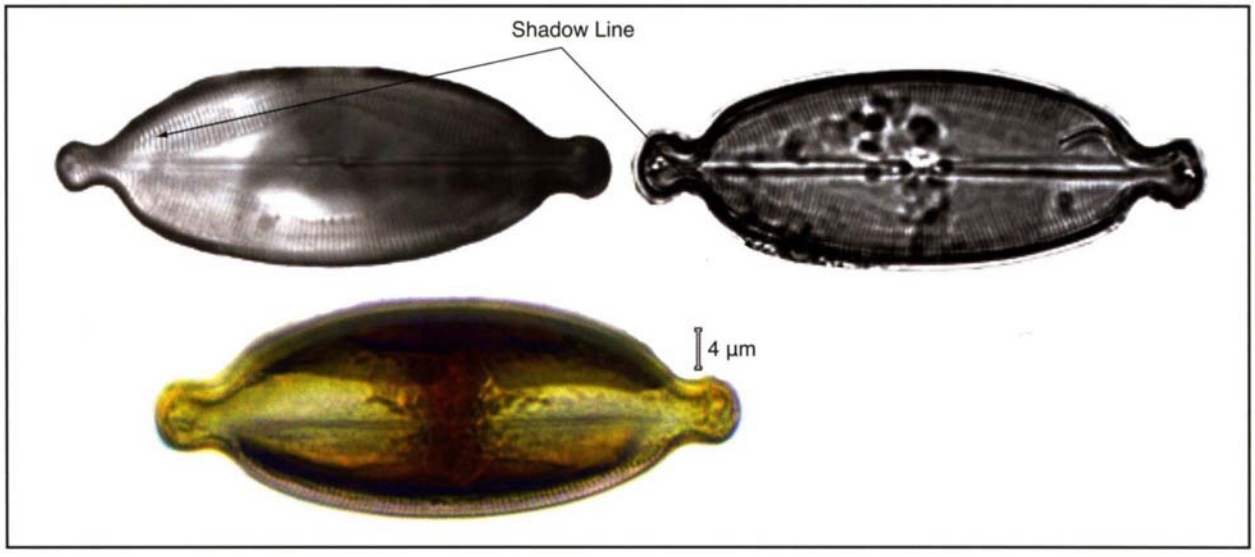

Figure 10-65 *Caloneis*

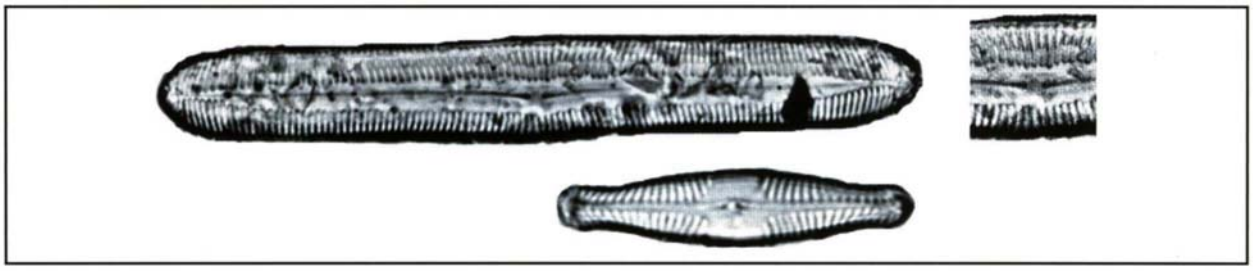

Figure 10-66 *Pinnularia*

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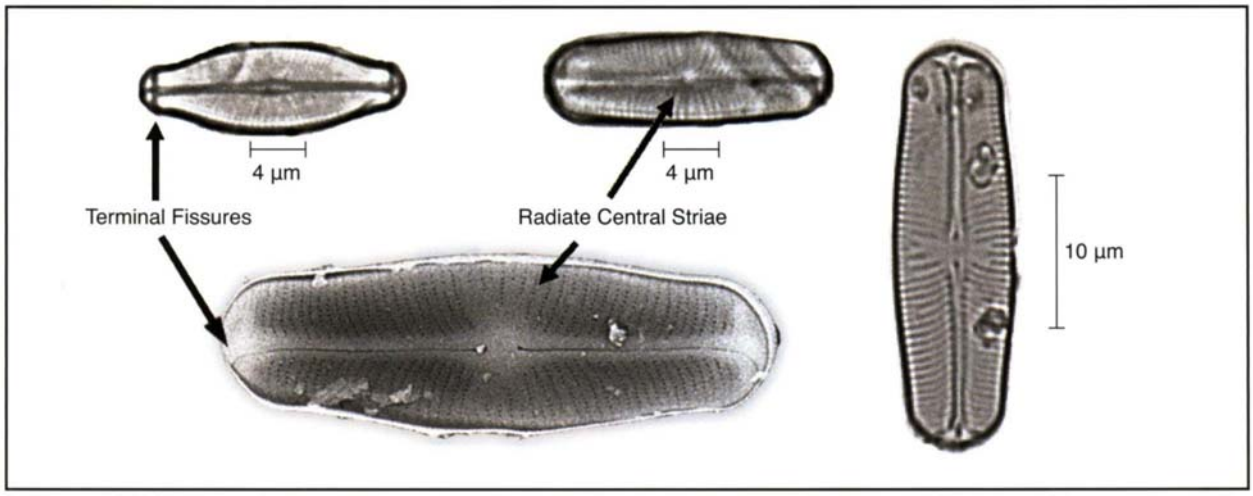

Figure 10-67 *Sellaphora*

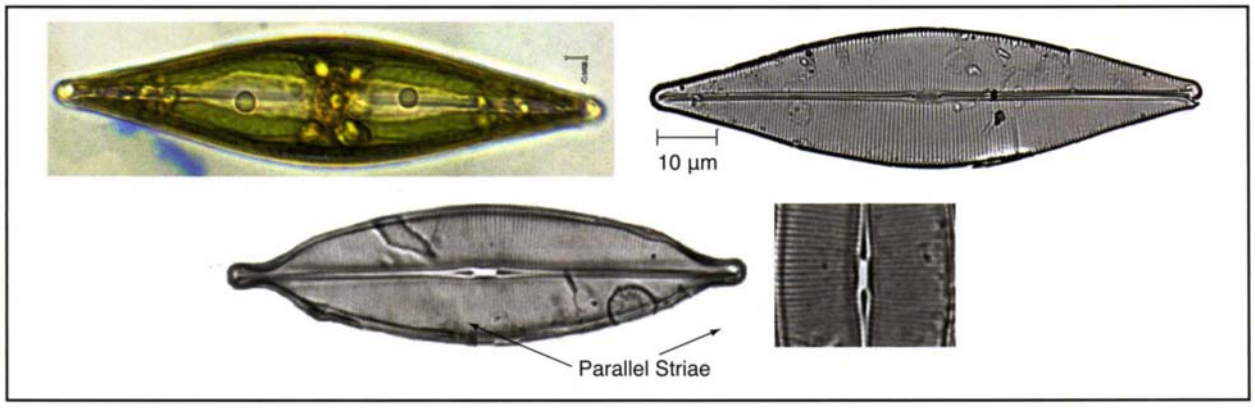

Figure 10-68 *Craticula*

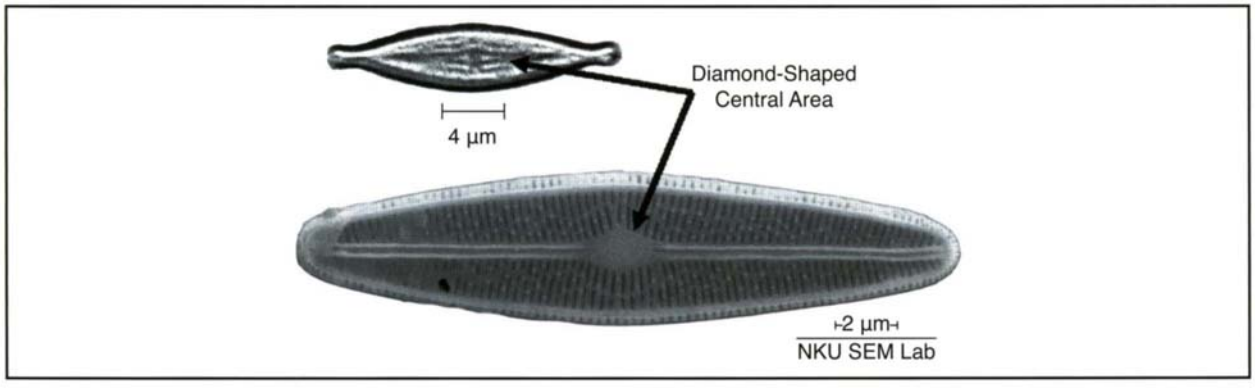

Figure 10-69 *Brachysira*

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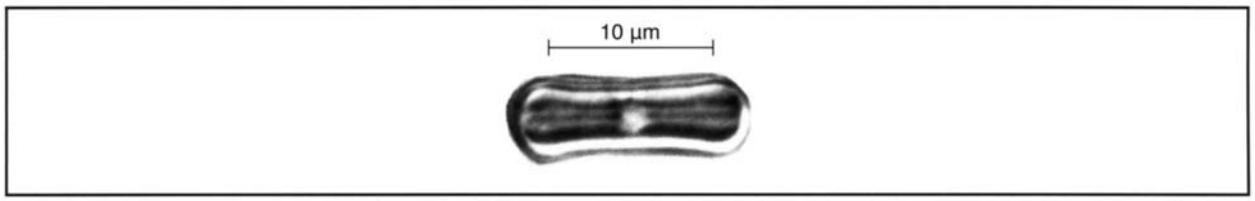

Figure 10-70 *Diadesmis*

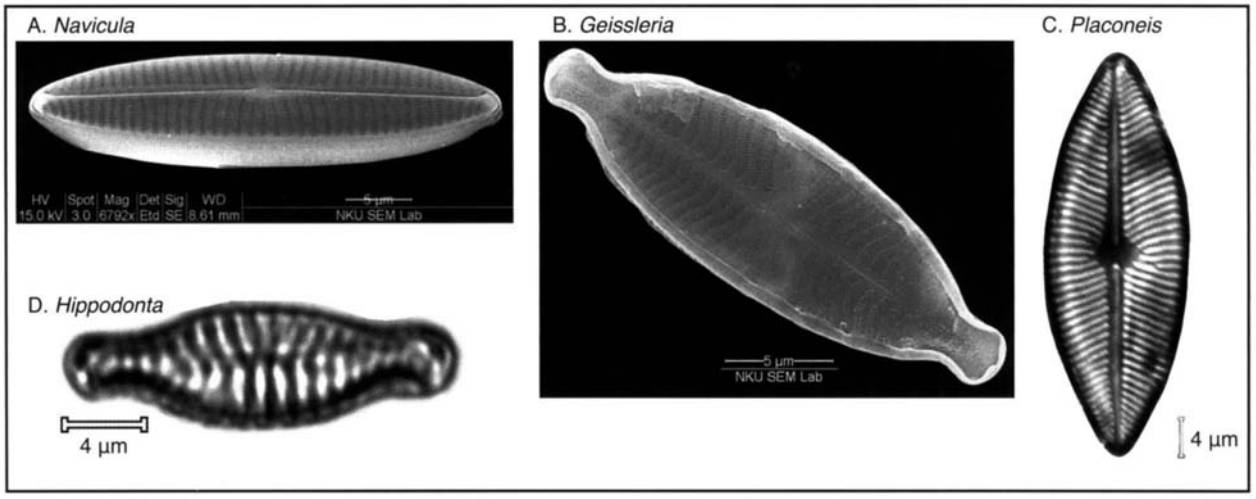

Figure 10-71 *Navicula* and related genera

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Our knowledge of diatoms, their taxonomy, and their value as ecological indicators owes much to the pioneering work of Ruth Patrick, mentor of Miriam Steinitz Kannan. We thank her for her teachings and inspiration that make it possible for this knowledge to be passed on to future generations of students. Much of the material in this chapter was produced as part of a manuscript "The Ohio River Diatoms: An Atlas of Common Species and Their Application to River Ecology." That guide is based on samples collected during a multiuniversity study of the Ohio River known as River Run, and in particular River Run 2001. The authors would like to thank all the member of the River Run 2001 team who helped collect the diatom samples: Dr. John Hageman, Dr. Michael C. Miller, Dr. Charles Sommerville, Lisa Smith, and Joseph Van Skaik. Funding for the fieldwork was provided by ORBCRE (Ohio River Basin Consortium for Research and Education), Cinergy, Ingram Barge Company, Bass Pro Shops, the Northern Kentucky University Center for Integrative Natural Sciences and Mathematics (CINSAM), and the parent departments of the participating universities' faculty. Funding for diatom laboratory work was provided to Joshua T. Cooper by CINSAM and by two Northern Kentucky University Greaves Undergraduate Research Fellowships. Scanning electron micrographs were provided with the assistance of Karl Hagglund (Northern Kentucky University SEM operator). We owe special thanks to Patricia Klonicki and Vicki Martin Kier for their editorial comments and many helpful suggestions.

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REFERENCES

Adl, S.M., A.G.B. Simpson, M.A. Farmer, R.A. Andersen, O.R. Anderson, J.R. Barta, S.S. Bowser, G. Brugerolle, R.A. Fensome, S. Fredericq, T.Y. James, S. Karpov, P. Kugrens, J. Krug, C.E. Lane, L.A. Lewis, J. Lodge, D.H. Lynn, D.G. Mann, R.M. McCourt, L. Mendoza, Ø. Moestrup, S.E. Mozley-Standridge, T.A. Nerad, C.A. Shearer, A.V. Smirnov, F.W. Spiegel, and M.F.J.R. Taylor. 2005. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *Jour. Eukary. Microbiol.*, 52(5):399–451.

American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF). 2005. *Standard methods for the examination of water and wastewater.* 21st ed. Washington, D.C.: APHA.

Bates, S.S., D.L. Garrison, and R.A. Horner. 1998. Bloom dynamics and physiology of domoic-acid-producing *pseudo-nitzschia* species. In *Physiological ecology of harmful algal blooms.* D.M. Anderson, A.D. Cembella, and G.M. Hallegraeff, eds. Heidelberg: Springer-Verlag.

Battarbee, R.W. 1986. Diatom analysis. In *Handbook of holocene palaeoecology and paleohydrology.* B.E. Berglund, ed. New York: John Wiley & Sons.

Camburn, K.E., and D.F. Charles. 2000. *Diatoms of low-alkalinity lakes in the northeastern United States.* Special Publication 18. Philadelphia: The Academy of Natural Sciences of Philadelphia.

Collins, G.B., and R.G. Kalinsky. 1977. *Studies on Ohio diatoms: Diatoms of the Scioto River basin.* Columbus, Ohio: Ohio State University.

Compère, P. 2000. Clé provisoire pour la détermination des genres de diatomées d'eau douce [Tentative key for the determination of freshwater diatom genera]. Available online: www.br.fgov.be/RESEARCH/EDITION/keydiato_BR.html.

Cox, E.J. 1996. *Identification of freshwater diatoms from live material.* London: Chapman & Hall.

Dickman, D.M. 1998. Benthic marine diatom deformities associated with contaminated sediments in Hong Kong. *Environ. Int.*, 24(7):749–759.

Dickman, M.D., J.R. Yang, and I.D. Brindle. 1990. Impacts of heavy metals on higher aquatic plant, diatom and benthic invertebrate communities in the Niagara River Watershed near Welland, Ontario. *Water Poll. Res. Jour. Can.*, 25(2):131–159.

Dixit, S.S, J.P. Smol, J.C. Kingston, and D.F. Charles. 1992. Diatoms: Powerful indicators of environmental change. *Environ. Sci. Technol.*, 26(1):23–33.

Fritz, S.C., S. Juggins, R.W. Battarbee, and D.R. Engstrom. 1991. Reconstruction of past changes in salinity and climate using a diatom-based transfer function. *Nature*, 352:706–708.

Gordon, R., and R.W. Drum. 1970. A capillarity mechanism for diatom gliding locomotion. *Proc. Nat. Acad. Sci.*, 67(1):338–344.

Hustedt, F. 1930. *Die susswasser-flora mitteleuropas.* Jena, Germany: Verlag von Gustaf Fisher.

Hustedt. F. 1985. *The pennate diatoms: A translation of Hustedt's "Die Kieselalgen, 2. Teil."* Translated by N.G. Jensen. Koenigstein. Germany: Koeltz Scientific Books.

Kingston, J.C. 2003. Araphid and monoraphid diatoms. In *Freshwater algae of North America: Ecology and classification.* J.D. Wehr and R.G. Sheath, eds. San Diego: Academic Press.

Kociolek, J.P., and S.A. Spaulding. 2003a. Symmetrical naviculoid diatoms. In *Freshwater algae of North America: Ecology and classification.* J.D. Wehr and R.G. Sheath, eds. San Diego: Academic Press.

Kociolek, J.P., and S.A. Spaulding. 2003b. Eunotioid and asymmetrical naviculoid diatoms. In *Freshwater algae of North America: Ecology and classification.* J.D. Wehr and R.G. Sheath, eds. San Diego: Academic Press.

Krammer, K., and Lange-Bertalot, H. 1986. Bacillariophyceae. 1. Teil: Naviculaceae. In *Süsswasserflora von mitteleuropa.* H. Ettl, J. Gerloff, H. Heynig, and D. Mollenhauer, eds. New York: Gustav Fischer Verlag.

Krammer, K., and Lange-Bertalot, H. 1988a. Bacillariophyceae. 2. Teil: Bacillariaceae, Epithemiaceae, Surirellaceae. In *Süsswasserflora von mitteleuropa.* H. Ettl, J. Gerloff, H. Heynig, and D. Mollenhauer, eds. New York: Gustav Fischer Verlag.

Krammer, K., and Lange-Bertalot, H. 1988b. Bacillariophyceae. 3. Teil: Centrales, Fragilariaceae, Eunotiaceae. In *Süsswasserflora von mitteleuropa.* H. Ettl, J. Gerloff, H. Heynig, and D. Mollenhauer, eds. New York: Gustav Fischer Verlag.

Lange-Bertalot, H., and G. Moser. 1994. Brachysira: Monographie der Gattung. In *Bibliotheca diatomologica*, Band 29. Berlin: Verlag J. Cramer.

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Li, C.-W., and E. Volcani. 1984. Aspects of silicification in wall morphogenesis of diatoms. In *Philosophical transactions of the Royal Society of London. Series B, biological sciences.* Vol. 304, No. 1121, Mineral Phases in Biology. London: The Royal Society.

Lobo, E.A. 1997. Approaches for evaluation of river water pollution using epiphytic diatom assemblages. *Diatom*, 13:105–111.

Lowe, R. 1974. *Environmental requirements and pollution tolerance of freshwater diatoms.* Cincinnati, Ohio: USEPA.

Lowe, R.L. 2003. Keeled and canalled raphid diatoms. In *Freshwater algae of North America: Ecology and classification.* J.D. Wehr and R.G. Sheath, eds. San Diego: Academic Press.

Mann, D.G. 1996. Chloroplast morphology, movements and inheritance in diatoms. In *Cytology, genetics and molecular biology of algae.* B.R. Chaudhary and S.B. Asgrawal, eds. Amsterdam: SPB Academic Publishing.

Palmer, C.M. 1962. Algae in water supplies. US Public Health Service Publication No. 657. Washington D.C.: US Department of Health, Education, and Welfare.

Palmer, C.M. 1977. Algae and water pollution. EPA-600/9-77-036. Cincinnati, Ohio: USEPA.

Patrick R. 1963. The structure of diatom communities under varying ecological conditions. *Ann. N.Y. Acad. Sci.*, 108:359.

Patrick, R., and C.W. Reimer. 1966. *The diatoms of the United States: Exclusive of Alaska and Hawaii.* Vol. 1. Philadelphia: The Academy of Natural Sciences of Philadelphia.

Patrick, R., and C.W. Reimer. 1975. *The diatoms of the United States: Exclusive of Alaska and Hawaii.* Vol. 2. Philadelphia: The Academy of Natural Sciences of Philadelphia.

Pickett-Heaps, J.D., and Pickett-Heaps, J. 2003. Teacher's guide. *Diatoms: Life in glass houses.* DVD. Melbourne: Cytographics. Available online: www.cytographics.com/resource/catalog/tapes/in-dia.pdf.

Prechtl, J., C. Kneip, P. Lockhart, K. Wenderoth, and U. Maier. 2004. Intracellular spheroid bodies of *Rhopalodia gibba* have nitrogen-fixing apparatus of cyanobacterial origin. *Mol. Biol. Evol.* 21(8):1477–1481

Rangel, M., E.L. Malpezzi, S.M. Susini, and J.C. de Freitas. 1997. Hemolytic activity in extracts of the diatom *Nitzschia. Toxicon*, 35(2):305–309.

Round, F.E., and D.G. Mann. 1981. The diatom genus *Brachysira.* I: Typification and separation from *Anomoeoneis. Arch. Protistenkunde*, 124:221–231.

Round, F.E., R.M. Crawford, and D.G. Mann. 1990. *The diatoms: Biology and morphology of the genera.* New York: Cambridge University Press.

Ruggiu, D., A. Luglié, A. Cattaneo, and P. Panzani. 1998. Paleoecological evidence for diatom response to metal pollution in Lake Orta (N. Italy). *Jour. Paleolimnol.*, 20(4):333–345.

Schmid, A.M. 1979. Wall morphogenesis in diatoms: The role of microtubules during pattern formation. *Eur. Jour. Cell Biol.*, 20:125.

Sheath, R.C., and J.D. Wehr. 2003. Introduction to freshwater algae. In *Freshwater algae of North America: Ecology and classification.* J.D. Wehr and R.C. Sheath, eds. San Diego: Academic Press.

Siver, P.A., P.B. Hamilton, K. Stachura-Suchoples, and J.P. Kociolek. 2005. *Diatoms of North America: The freshwater flora of Cape Cod.* Iconographia Diatomologia. Königstein, Germany: A.R.G. Gantner Verlag.

Smol, J.P., R.W. Battarbee, R.B. Davis, and J. Meriläinen (eds.) 1986. *Diatoms and lake acidity.* Developments in Hydrobiology Series. Dordrecht: W. Junk Publishers.

Steinitz-Kannan, M. 2005. Effects of the environment on morphology of the silica cell wall in freshwater diatoms. *Polym. Prepr.*, 46(1):27.

Stevenson, R.J., and Y. Pan. 1999. Assessing environmental conditions in rivers and streams with diatoms. In *The diatoms: Applications for the environmental and earth sciences.* E. Stoermer and J. Smol, eds. New York: Cambridge University Press.

Stevenson, R.J., M.L. Bothwell, and R.L. Lowe. 1996. *Algal ecology: Freshwater benthic ecosystems.* San Diego: Academic Press.

Stoermer, E.F., and M.L. Julius. 2003. Centric diatoms. In *Freshwater algae of North America: Ecology and classification.* J.D. Wehr and R.G. Sheath, eds. San Diego: Academic Press.

Thamatrakoln, K., and M. Hildebrand. 2007. Analysis of *Thalassiosira pseudonana* silicon transporters indicates distinct regulatory levels and transport activity through the cell cycle. *Eukary. Cell*, 6(2): 271–279.

Ussing, A.P., R. Gordon, L. Ector, K. Buczko, A.G. Desnitskiy, and S.L. Vanlandingham. 2005. The colonial diatom "Bacillaria paradoxa": Chaotic gliding motility, Lindenmeyer model of colonial morphogenesis,

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and bibliography, with translation of O.F. Müller (1783) "About a peculiar being in the beach water." In *Diatom monographs.* Andrzej Witkowski, ed. Koenigstein, Germany: Koeltz Scientific Books.

Van Dam, H., A. Mertens, and J. Sinkeldam. 1994. A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. *Neth. Jour. Aquat. Ecol.*, 28:117–133.

Van Heurck, H. 1896. *A treatise on the diatomaceae.* Translated by W.E. Baxter. London: Wesley & Son. Reprint, Berlin: Verlag J. Cramer, 1962.

Verb, R.G., and M.L. Vis. 2000. Comparison of benthic diatom assemblages from streams draining abandoned and reclaimed coal mines and nonimpacted sites. *Jour. N. Am. Bentholog. Soc.*, 19(2):274–288.

Wang, Y., J. Lu, J.-C. Mollet, M.R. Gretz, and K.D. Hoagland. 1997. Extracellular matrix assembly in diatoms (Bacillariophyceae). *Plant Physiol.*, 113:1071–1080.

Williams, D.M., and F.E. Round. 1987. Revision of the genus *Fragilaria. Diatom Res.*, 2:267–288.

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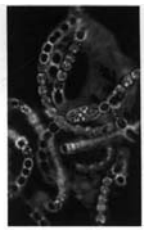

Chapter 11

Chrysophyta

Robert A. Andersen

GENERAL DESCRIPTION

The division Chrysophyta was assembled by Pascher (1914), who placed emphasis on the small size and the putative silica metabolism for diatoms (Bacillariophyceae), golden-brown algae (Chrysophyceae), and yellow-green algae (Xanthophyceae). Cell and molecular biology studies have showed that this assemblage is artificial, and consequently the original Chrysophyta is no longer recognized (e.g., Andersen 1987, Andersen and Preisig 2002, Preisig and Andersen 2002). The Chrysophyceae known to Pascher were also artificial, and several independent groups have been established. That is, the Haptophyceae were removed from the Chrysophyceae by Christensen (1962) based on the electron microscopic work of Parke, Manton, and colleagues (e.g., Parke et al. 1955; see also Hibberd 1976, Edvardsen et al. 2000). The silicoflagellates were placed in the Dictyochophyceae (Silva 1980), and cell/molecular studies brought the pedinellids (Moestrup 1995) and *Rhizochromulina* (O'Kelly and Wujek 1995) into that class (Saunders et al. 1995). Next, *Synura*, *Mallomonas*, and related taxa were placed in the Synurophyceae (Andersen 1987, 1989b); however, phylogenetically, this class remains closely related to the Chrysophyceae (e.g., Ariztia et al. 1991; Bhattacharya et al. 1992; Leipe et al. 1994; Saunders et al. 1995, 1997a; Lavau et al. 1997; Bailey et al. 1998; Andersen et al. 1999; Andersen 2007; Kristiansen and Preisig 2007). Many marine chrysophytes were removed and placed in the Pelagophyceae because of their distinctive features (Andersen et al. 1993, Saunders et al. 1997b), and still others were placed in the Chrysomerophyceae (Cavalier-Smith et al. 1995). A group of mostly freshwater chrysophytes was placed in the Phaeothamniophycae (Bailey et al. 1998) based on ultrastructure, pigmentation, and molecular phylogenetic analysis. And, just recently, a marine amoeboid organism called *Synchroma* was placed in the Synchromophyceae (Horn et al. 2007), which is closely related to the Chrysophyceae and Synurophyceae. In addition, the Bicosoecida (Grassé 1926) and the choanoflagellates, which Bourrelly (1957) included in the Chrysophyceae, are not considered here. Therefore, the Chrysophyta established nearly 100 years ago by Pascher is no longer recognized, and in its place, today's Chrysophyta contains a smaller Chrysophyceae as well as the Synurophyceae (and possibly the Synchromophyceae).

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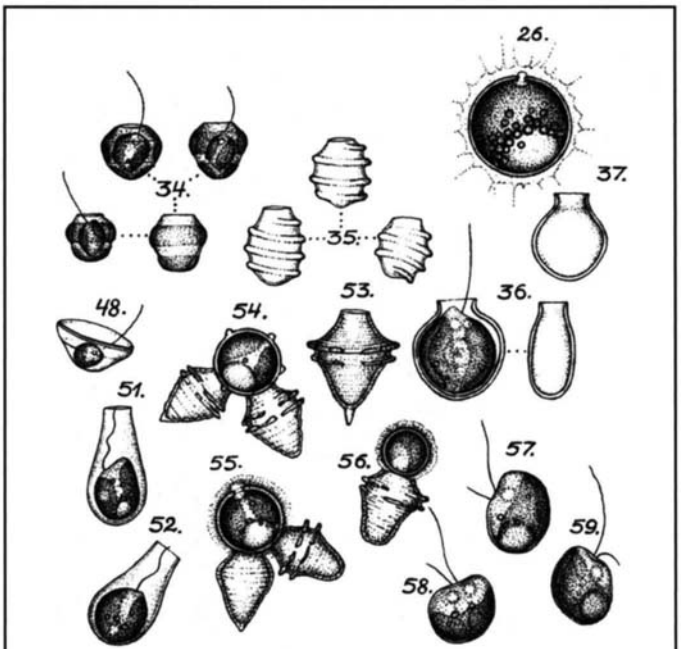
BIOLOGY

Both the Chrysophyceae and Synurophyceae occur predominantly in freshwater. They live in a wide range of freshwater environments from polar regions to the tropics, but most commonly occur in cold temperate lakes, ponds, and bogs. A few species of both classes are reported from marine environments; for the Chrysophyceae, a few soil and snow algae are known. Many Chrysophyceae are free-swimming, single-cell flagellates or motile colonies; however, amoeboid (rhizopodial), plasmodial, palmelloid (capsoid), coccoid, and parenchymatous forms also occur (see Bourrelly 1968/1981, Starmach 1985, Preisig and Andersen 2002) (Figures 11-1, 11-2, 11-3). The Synurophyceae are free-swimming single-celled or colonial flagellates during some or all of their life history (see Andersen 1987, 1989b; Andersen and Preisig 2002; Kristiansen and Preisig 2007) (Figures 11-4 and 11-5). Benthic palmelloid stages are known (e.g., Pascher 1912, Andersen 1989b), and the benthic stage may be an important, unrecognized ecological phase that contributes to bloom development.

The siliceous cyst (= statospore, stomatocyst) is the single unique synapomorphy for the Chrysophyceae and Synurophyceae, although Sandgren (1980b, 1989) reports that cyst development and morphogenesis are distinct for each class. Cysts are apparently produced as a result of sexual reproduction or from a vegetative cell without any sexual fusion. Cysts are formed endogenously as silica is deposited into the silica deposition vesicle (see Preisig 1986, 1994), and they vary significantly in morphology,



26 = Cyst of *Monochrysis maior*
34 = *Kephyrion littorale*
35 = *Kephyrion spirale*
36–37 = *Kephyrion boreale*
48 = *Kephyrion cupuliforme*
51–52 = *Chrysococcocystis ampulla*
53–56 = *Stenokalyx inconstans* (Note the cyst formation resulting from sexual [54, 55] and asexual [56] events.
57–59 = *Ochromonas obliqua*

Figure 11-1 Chrysophyceae illustrations from Skuja (1956)

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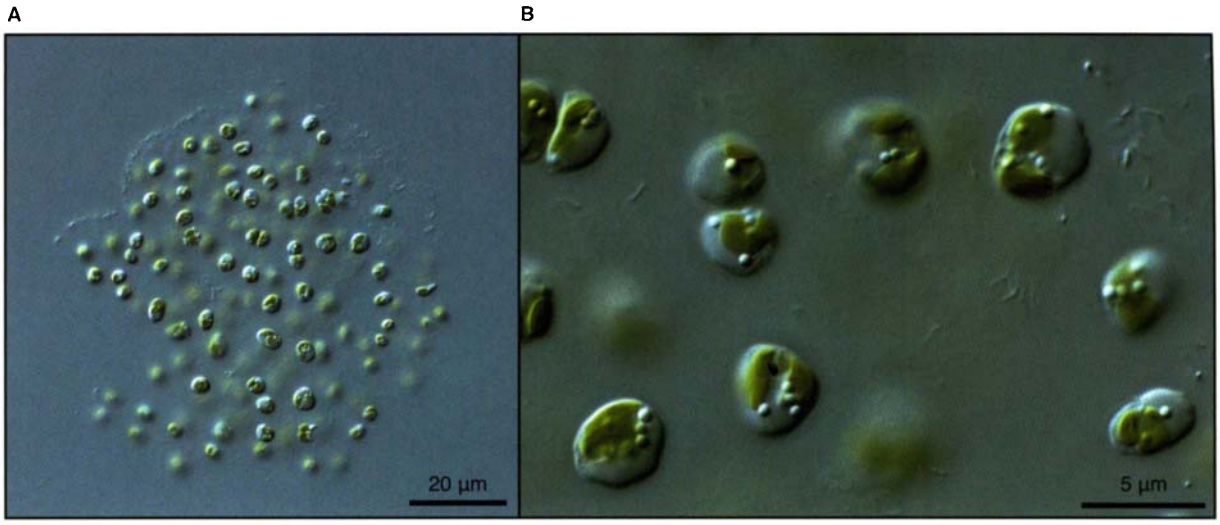

Figure 11-2 *Chrysocapsa vernalis* showing the capsoid nonmotile colony (A) and individual cells (B)

Figure 11-3 The amoeboid *Chrysamoeba pyrenoidifera* cells showing the many pseudopods used to capture bacteria

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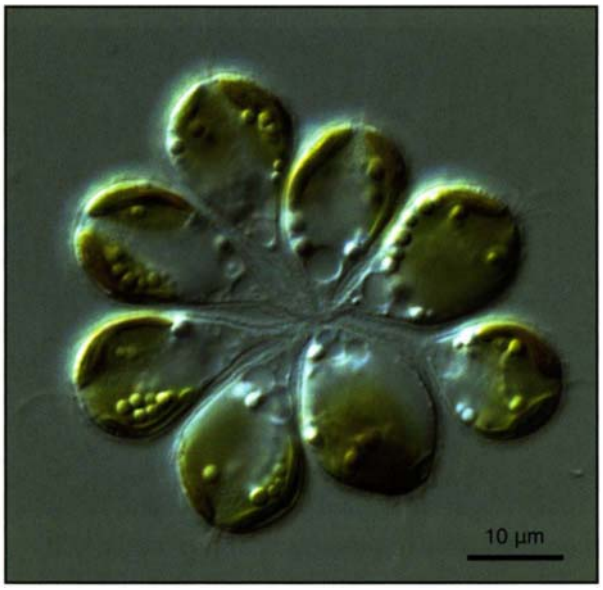

Figure 11-4 A colony of *Synura petersenii* showing the chloroplasts, nucleus, contractile vacules, and flagella

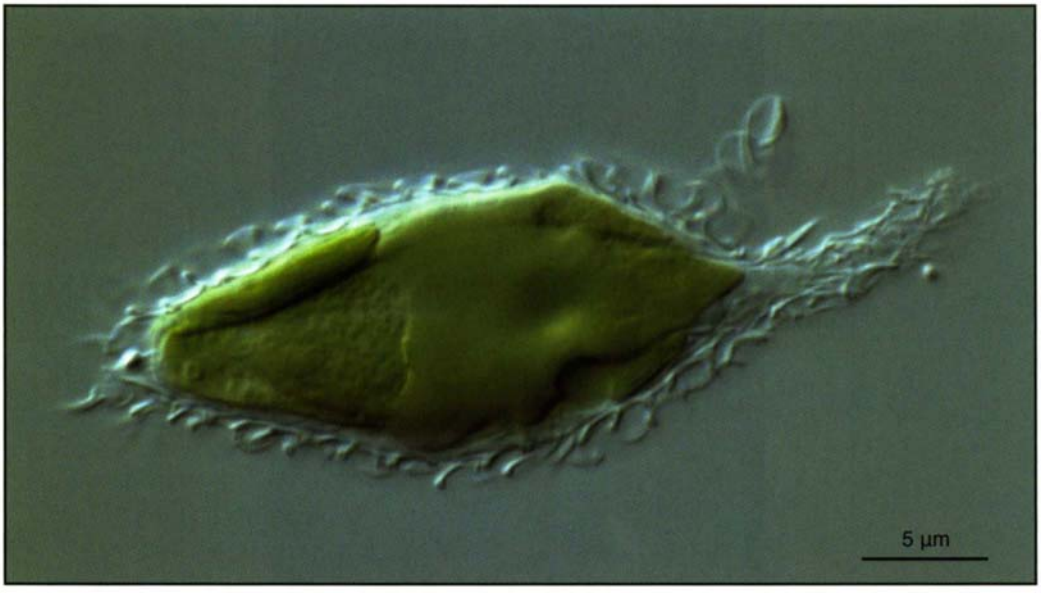

Figure 11-5 *Mallomonas insignis* cell showing the two plastids, the nucleus, the posterior contractile vacuoles, and the silica-scale covering

from smooth and spherical to oval and highly ornamented (Figure 11-6). All cysts have a narrow pore that is closed at maturity with an organic plug. Many cysts are known from sediments, and these have been described based upon their morphology (e.g., Sandgren 1980a, 1980b, 1981, 1983, 1989, 1991; Duff et al. 1994, 1995; Leadbeater and Barker 1995).

The flagellate cells of the Chrysophyceae have two obliquely inserted heterodynamic flagella of unequal length (Vlk 1931, Bourrelly 1957, Leadbeater 1989). The longer, immature flagellum is directed anteriorly, and it beats in a sine wave. This flagellum has two rows of tripartite hairs, or mastigonemes (Bouck 1972, Bouck and Brown 1973), and these hairs reverse the thrust so that the flagellum pulls rather than pushes

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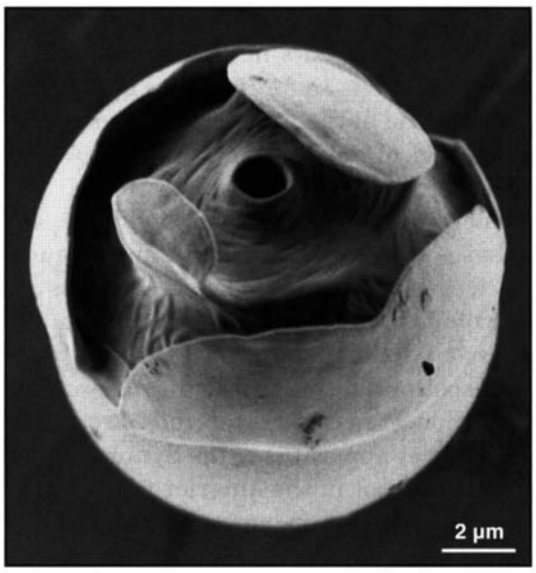

Figure 11-6 A siliceous cyst of *Ochromonas* cf. *gloeopara*. Note the small pore opening from which the living cell escapes during excystment. There are two sets of flanges on the anterior end of the cyst

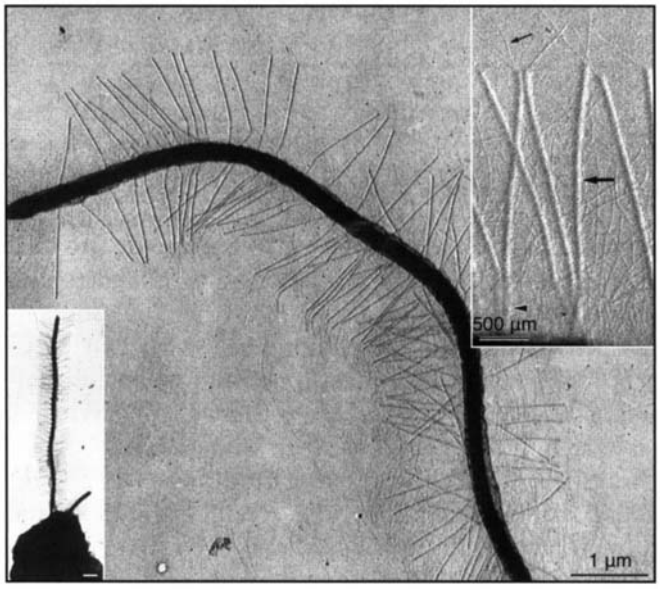

Figure 11-7 Shadow-cast preparation demonstrating the tripartite flagellar hairs of *Ochromonas*. Lower left inset shows the whole cell and both flagella (scale bar = 1 µm). The main figure shows an enlarged view of the immature flagellum and its hairs. Scale bar = 1 µm. The upper right inset shows the fine details of the tripartite structure: arrowhead = base; large arrow = shaft; small arrow = terminal hairs.

the cell (Sleigh 1964, 1974) (Figure 11-7). The shorter, mature flagellum is directed laterally when it is present. The colonial Synurophyceae have two subequal flagella (*Chrysodidymus*, *Pseudosyncrypta*, *Synura*, *Tessellaria*), and some species of *Mallomonas* (formerly classified as *Mallomonopsis*) also have two subequal flagella. For the other single-celled Synurophyceae, each cell has two very unequal flagella or a single flagellum with a barren second basal body (Bourrelly 1968/1981; Starmach 1985). Like

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most heterokont algae, the longer, immature flagellum of the Synurophyceae bears tripartite tubular hairs and beats in a sine wave. The shorter flagellum, when present and visible, is less active. In *Mallomonas guttata*, unusual appendages have been observed at the distal end of the shorter flagellum (Zhang et al. 1990a). Small organic scales adorn the surface of the flagella (Hibberd 1973, Zimmermann 1977).

Swimming cells of both classes are phototactic, and they each bear a photoreceptor apparatus to detect light. In the Chrysophyceae, a photoreceptor-related swelling occurs near the base of the short flagellum, and frequently this swelling is associated with an orange-red eyespot (stigma). The eyespot is usually a single layer of carotenoid-containing lipid globules at the edge of a chloroplast, and this region is pressed closely against the depression in the surface of the cell into which fits the swelling on the short flagellum. The eyespot and flagellar swelling are commonly referred to collectively as the photoreceptor apparatus (see Foster and Smyth 1980, Kreimer 1994, Walne et al. 1995). The photoreceptor apparatus of the Synurophyceae is completely different: they do not possess a red eyespot in the anterior margin of the chloroplast, and the photoreceptor consists of a swelling on one or both of the flagella (Andersen 1985, 1989b). Some species of *Synura* (e.g., *S. curtispina* [Petersen and Hansen 1956, Asmund 1968]) and *S. sphagnicola* occasionally have red droplets in the anterior end of the cells, but these droplets are not associated with the chloroplast (Hibberd 1978). *Chrysodidymus synuroideus* also has red droplets but these are located in the cell posterior (Graham et al. 1993).

The photosynthetic Chrysophyceae usually have one or two parietal chloroplasts, with or without pyrenoids. The chloroplast lamellae typically have three thylakoids, and a girdle lamella is usually present. The chloroplast is surrounded by two membranes of endoplasmic reticulum that is confluent with the outer membrane of the nuclear envelope. Pyrenoids, when present, are often immersed and barely detectable with the light microscope. Ultrastructurally, the pyrenoid matrix is usually free of thylakoids. The nonphotosynthetic Chrysophyceae are phagotrophic (see Preisig et al. 1991); however, many photosynthetic Chrysophyceae are mixotrophic (i.e., they photosynthesize and they capture particulate food by phagotrophy).

The Synurophyceae are reported to have two chloroplasts; however, Harris (1953) showed the presence of a single chloroplast with two very deeply divided lobes for 25 species of *Mallomonas*. The plastid number and condition has never been carefully reexamined in these taxa, and if there are two deeply divided lobes of a single plastid, it is not obvious using either light or electron microscopy. The chloroplast is surrounded by two membranes of endoplasmic reticulum, which is typically not confluent with the outer membrane of the nuclear envelope (Wujek and Kristiansen 1978; Andersen 1987, 1989b; Zhang et al. 1990b; Pipes et al. 1991; Graham et al. 1993). Pyrenoids are rare in the Synurophyceae, but they have been reported for *Synura sphagnicola* (Hibberd 1978). Two colorless species of *Mallomonas* have been described, but their existence is questioned (see Preisig et al. 1991).

The chloroplasts of the Chrysophyceae are yellow-brown or yellow-green, and they contain chlorophylls *a*, c_1, and c_2 (Andersen and Mulkey 1983, Jeffrey 1989). Note that chlorophylls c_1 and c_2 are actually chlorophyllides because they lack a phytol tail. The major carotenoid pigments are fucoxanthin, violaxanthin, antheraxanthin, neoxanthin, zeaxanthin, and β-carotene (Withers et al. 1981, Bjørnland and Liaaen-Jensen 1989). In *Hibberdia*, both fucoxanthin and antheraxanthin function as light-harvesting pigments (Alberte and Andersen 1986). The Synurophyceae have chlorophyll *a* and c_1 but they lack chlorophyll c_2 (Andersen and Mulkey 1983, Jeffrey 1989). The lack of chlorophyll c_2 distinguishes the Synurophyceae from the Chrysophyceae and other heterokont algae. A new form of chlorophyll *c* has been reported

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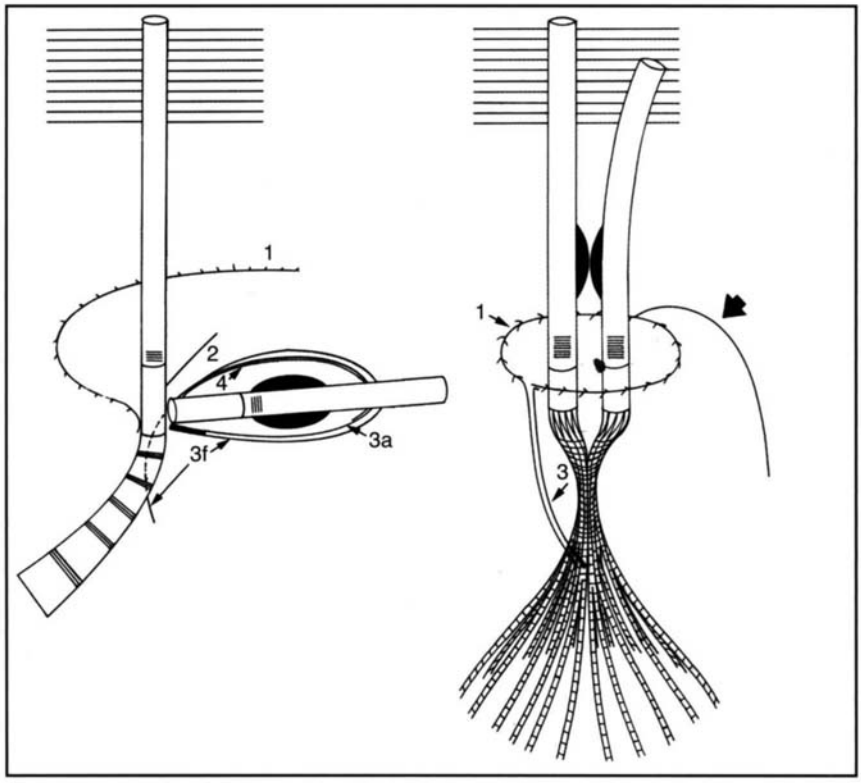

Figure 11-8 General illustrations of the flagella, microtubular roots, and rhizoplasts for the Chrysophyceae (A) and Synurophyceae (B). Numbers refer to flagellar roots, and numbers with letters refer to the specific microtubule in the flagellar root. Large arrow indicates a cytoskeletal microtubule that is nucleated from the R_1 root of *Synura*. Numerous cytoskeletal microtubules are present in both chrysophycean and synurophycean cells (indicated by very short lines along the R_1 root).

for *Synura petersenii* (Fawley 1989), but the chemical structure of this new pigment has not been determined and the extent of its occurrence in the Synurophyceae is not known. The carotenoid pigments are similar to those in the Chrysophyceae (Bidigare and Andersen, unpublished observations).

The chloroplast genome of *Synura petersenii* is only 91.5 kb (Wee et al. 1993), one of the smallest chloroplast genomes known (see Coleman and Goff 1991). Another member of the class, *C. synuroideus* Prowse, 1962, has a chloroplast genome size of 102 kb (Graham et al. 1993), and these data suggest that the chloroplast genome size of the Synurophyceae is quite small compared to other algae and plants. The organization of the plastid genome in *Synura* is scattered rather than as a ring (Coleman 1985). The mitochondrial genome of *C. synuroideus* is 33 kb (Graham et al. 1993) and the putative mitochondrial genome of *S. petersenii* is 34.5 kb (Wee et al. 1993); these mitochondrial genomes are comparable in size to those of other algal groups (see Coleman and Goff 1991). Other molecular biology details are summarized by Wee et al. (1996).

The basal body of the short flagellum in the Chrysophyceae usually lies at an angle of approximately 90° with respect to the long flagellum (Figure 11-8), but narrower or wider angles also occur [e.g., ca. 30° in *Chrysonebula* (Hydrurales), 160° in *Hibberdia* (Hibberdiales)]. The basal bodies are interconnected by fibrous bands. Also, a rhizoplast (= system II fiber) usually forms a connection between the basal apparatus and the nucleus (in *Chrysosphaerella,* it is directed toward the plasma membrane and attaches to a mitochondrion; see Andersen 1990). There are often four microtubular

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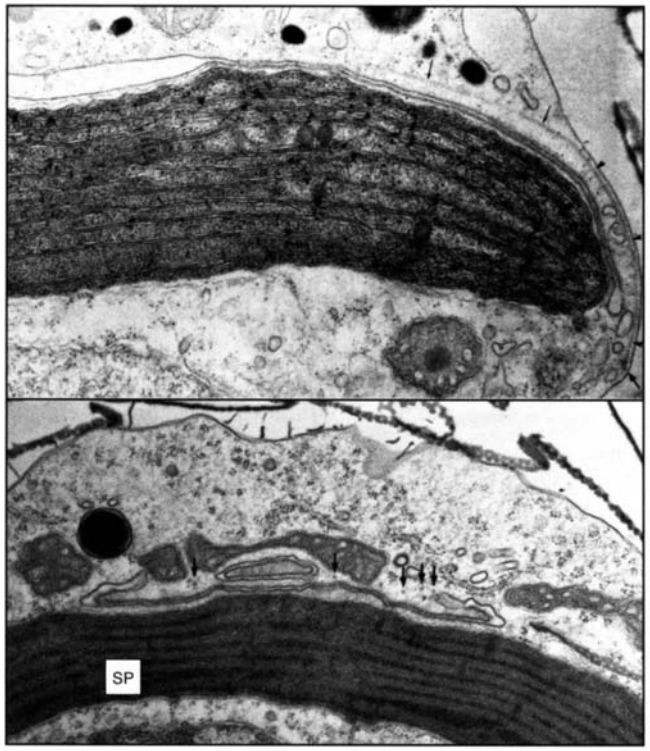

Figure 11-9 *Synura uvella* (top micrograph). Silica deposition vesicle (longitudinal section) that will give rise to a silica scale is shown along the outer margin of the chloroplast. Note the cytoskeletal microtubule that originates from the R_1 root (lower right corner). The cytoskeletal microtubule lies closely associated with the plasma membrane (arrowheads) before diverging toward the cell posterior (arrows). *Synura petersenii* (bottom micrograph). The silica deposition vesicle is in cross section, and several cytoskeletal microtubules (arrows) are shown in cross section. SP = scale-producing chloroplast.

roots (R_1, R_2, R_3, R_4), but a reduced number occurs in several species. Roots R_3 and R_4 often form a loop under the shorter flagellum (no such loop occurs in Hibberdiales and Hydrurales). From the surface of some roots (i.e., R_1, R_3), cytoplasmic microtubules can be nucleated, providing cytoskeletal support to shape the cell. For more information on the flagellar apparatus, see for example, Andersen (1989a, 1991) and Preisig (1989). For details of flagellar transformation, see Wetherbee et al. (1988).

The ultrastructure of the flagellar apparatus of Synurophyceae is not only different from the Chrysophyceae, but it is unique among all flagellate cells because two to four absolute orientations occur in the Synurophyceae (Andersen 1985, 1991). The absolute orientation of the flagellar apparatus consists of the arrangement of the flagellar apparatus with respect to other cellular organelles. The two basal bodies are arranged in a nearly parallel fashion, they are connected with two or more striated fibers, and in turn the basal apparatus is attached to the nucleus with one or two large rhizoplasts. Cells have either R_1 microtubular roots only, or R_1 and R_3 roots; the R_2 and R_4 microtubular roots that are typical for members of the Chrysophyceae are absent (Figure 11-8). The distinctive R_1 root is attached to the rhizoplast, and it extends anteriorly where it forms a loop around the flagella. Numerous cytoskeletal microtubules extend from this root, providing cytoskeletal support to shape the cell. Specialized cytoskeletal microtubules also extend down from the R_1 root along the chloroplasts, maintaining the chloroplasts in a fixed position. Additional specialized

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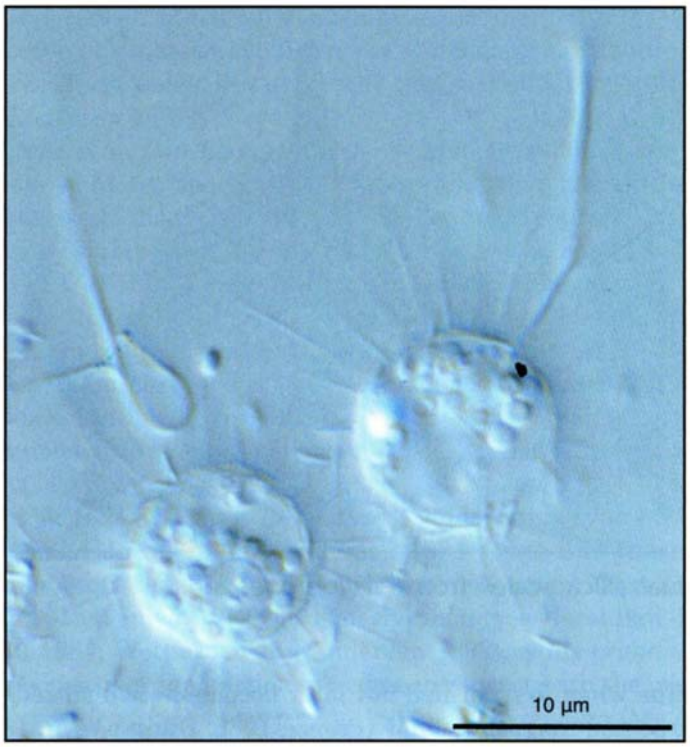

Figure 11-10 The colorless and phagotrophic *Paraphysomonas vestita* showing its silica scales.

cytoskeletal microtubules extend down along the outside of one chloroplast and are involved in silica scale formation (Figure 11-9) (Schnepf and Deichgräber 1969; Mignot and Brugerolle 1982; Andersen 1985, 1989b; Beech and Wetherbee 1990b; Pipes et al. 1991; Pipes and Leedale 1992; Graham et al. 1993).

The major carbohydrate storage product of the Chrysophyceae is leucosin (Klebs 1893, Archibald et al. 1963), chrysolaminaran (Quillet 1955, Beattie et al. 1961, Smestad-Paulsen and Myklestad 1978), or similar relatively small (dp <40) ß-1,3 linked glucan. The size is small, less than 40 sugar residues, and therefore the product affects cellular osmolarity and must be stored in a vacuole. The carbohydrate storage product is not known for the Synurophyceae, but it is assumed to be similar to that of the Chrysophyceae, diatoms, and other heterokont algae. Small lipid droplets are found in both the Chrysophyceae and Synurophyceae.

Many Chrysophyceae have naked cells (i.e., they have no cell covering). A few genera such as *Chrysosphaerella*, *Paraphysomonas*, *Polylepidomonas*, and *Spiniferomonas* have a cell covering of siliceous scales (Figure 11-10). Their scales are radially or biradially symmetrical, and they formed endogenously in silica deposition vesicles near the periphery of the cell that are not associated with the chloroplast endoplasmic reticulum (see Preisig and Hibberd 1982a, 1982b, 1983; Preisig 1986, 1994; Leadbeater and Barker 1995). The mature scales are extruded onto the cell surface where they are adhered in some unknown way. Unmineralized scales also occur in a small number of genera (e.g., *Chrysolepidomonas*; Peters and Andersen 1993a, 1993b). Several genera have cells surrounded by a lorica, which provides protection but does not allow for turgor pressurized cells as found in plants with cell walls. The loricas have an organic composition of protein, cellulose or chitin (Schnepf et al. 1975, Herth et al. 1977, Herth 1979, Herth and Zugenmaier 1979). Some loricas are produced from overlapping organic scales while others are spun as a continuous organic strand extruded from the cell. Loricae

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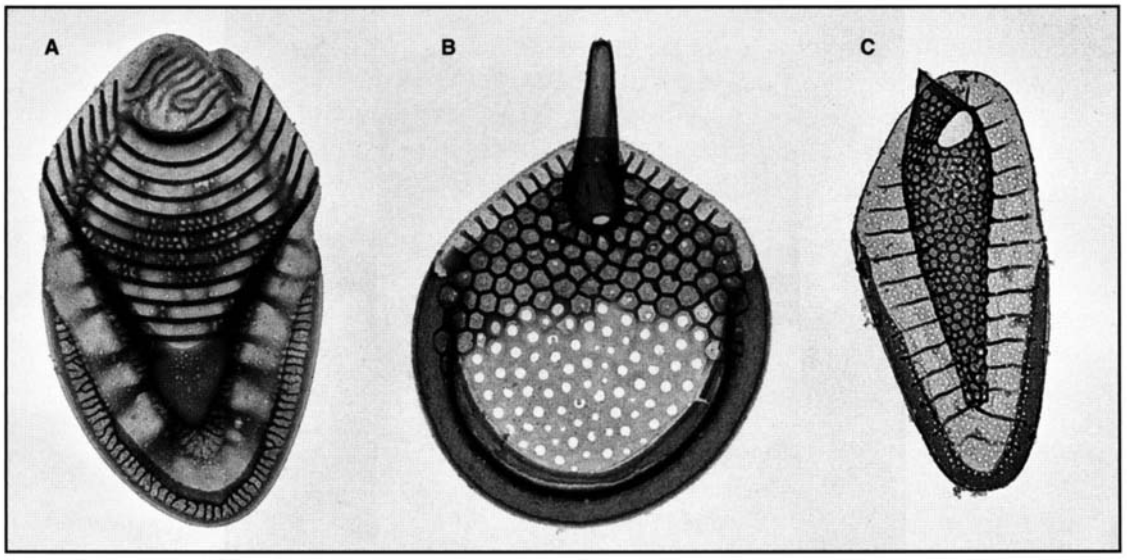

Figure 11-11 Individual silica scales from *Mallomonas striata* (A), *Synura spinosa* (B), *Synura petersenii* (C)

are hyaline when first formed but later become brownish-red due to incorporation of manganese and iron compounds (Preisig 1986, Dunlap et al. 1987).

The Synurophyceae are characterized by having bilaterally symmetrical silica scales (Figure 11-11). Silica scale morphology is used as the primary, and often only, character for identification of taxa (see Kristiansen and Preisig 2007). Gene sequences have been used to test the validity of this scale-based taxonomy (Lavau et al. 1997), and recent work suggests that the scale morphology may be conservative, masking hidden diversity in *S. petersenii* (Sung Min Boo, unpublished results). The scales are arranged in a highly organized fashion around each cell in most taxa. However, *Tessellaria volvocina* and *Synura lapponica* (rarely for *S. sphagnicola*) have scales that surround the colony rather than individual cells (Skuja 1956, Pipes et al. 1991, Goldstein et al. 2005, unpublished observations). Laboratory experiments have shown that scaleless cells can be produced under conditions of extreme silicon starvation (Sandgren and Barlow 1989, Leadbeater and Barker 1995, Sandgren et al. 1996), but these scaleless forms apparently never occur in nature. Scale formation, or scale biogenesis, has been described in some detail (see Schnepf and Deichgräber 1969; Mignot and Brugerolle 1982; Brugerolle and Bricheux 1984; Wetherbee et al. 1989, 1992, 1995; Preisig 1986, 1994; Leadbeater and Barker 1995; Ludwig et al. 1996; Wee 1997). The deployment of scales around the cell has also been described. In *Mallomonas adamas*, cells immediately following cell division have 30 scales (5 rows of 6 scales); scales are formed and secreted throughout interphase, and just before division, cells have 60 scales (5 rows of 12 scales). Scales are added singly, not in rows, and specific scale types are excreted and deployed to their specific locations (Lavau and Wetherbee 1994). In species such as *Mallomonas splendens*, scales have attached bristles (Beech et al. 1990). The bristles are excreted base-first, but the bristle is attached to the cell via a microtubule-enforced cytoplasmic strand, and once the bristle is completely excreted, the base of the bristle is pulled into place. Ecologically, scales and bristles are believed to play a role as anti-predatory devices against smaller predators (e.g., ciliates), but they seem to be ineffective against larger predators (Sandgren and Walton 1995).

Cells of the freshwater Chrysophyceae have one or more contractile vacuoles that are located the anterior end of the cell near the flagellar bases (Aaronson and Behrens 1974, Wessel and Robinson 1979, Aaronson 1993). The discharge mechanism is

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poorly understood, but a small membranous channel to the plasma membrane has been observed (Andersen 1982). Freshwater cells of the Synurophyceae have very similar contractile vacuoles; however, they are located posterior to the nucleus and chloroplasts (Andersen 1987).

Nutrition in the Chrysophyceae varies from obligate phototrophy to obligate phagotrophy. Many species are mixotrophic, that is, capable of photosynthesis as well as uptake of dissolved substances (osmotrophy) and food particles (phagotrophy). Osmotrophy is important for the uptake of sugars and amino acids (Pringsheim 1952, Hutner et al. 1953). *Ochromonas* and *Poterioochromonas* are considered sugar flagellates (*sensu* Doflein 1916) rather than acetate flagellates (*sensu* Pringsheim 1935). An exogenous source of vitamins is necessary for many chrysomonads, and osmotrophic uptake of vitamins has been demonstrated in *Ochromonas* and *Poterioochromonas* (Pringsheim 1952, Hutner et al. 1953). Alternatively, *Uroglena americana* must obtain its exogenous vitamins via phagotrophy of bacteria (Kurata 1986, Kimura and Ishida 1986). At least two types of phagotrophy have been reported; one involves the capture of food by flagella and the other by fine pseudopodia (Type 1 and Type 3 feeding mechanisms *sensu* Moestrup and Andersen 1991; Type 2 is characteristic of pedinellids, which have been transferred to the Dictyochophyceae.) Flagella actively draw particles to the cells that have the Type 1 mechanism (see Korshikov 1929, Sleigh 1964, Bird and Kalff 1987, Wetherbee and Andersen 1992), while organisms with Type 3 mechanisms rely passively on particles to come in contact with the pseudopodia (see Scherffel 1901). Type 1 feeding has been reported for many genera (see Moestrup and Andersen 1991, Holen and Boraas 1995). Particles are captured between the flagella and held for a short period of time while the cell apparently determines whether to accept or reject the food particle (see Wetherbee and Andersen 1992). If rejected, the particle is pushed away by the flagella, and the capture process begins again. If accepted, the cell produces a feeding basket, and the food particle is pushed into the basket by the flagella. In *Epipyxis*, the most thoroughly investigated organism, the feeding basket is formed and closed by movements of specific microtubules of the R_3 flagellar root (for details, see genus *Epipyxis* in this chapter, also Andersen and Wetherbee 1992). Type 3 feeding is less well studied. Food particles that are captured passively by pseudopodia move along the outer surface of the pseudopods and are drawn to the cell body. The engulfment process has not been described ultrastructurally for Type 3 feeding cells. Because many chrysomonads that produce pseudopodia are reported to lack flagella, it is unclear if the basal apparatus is present without emergent flagella and engulfment is controlled by flagellar root microtubules, or if there is a distinctly different phagotrophic process. *Chrysamoeba pyrenoidifera* bears flagella that have a typical *Ochromonas*-type flagellar apparatus (O'Kelly and Wujek 1995). Some pseudopods are supported by microtubules nucleated by the R_1 flagellar root, suggesting that it engulfs particles in a manner similar to *Epipyxis* (Andersen and Wetherbee 1992) or *Chrysosphaerella* (Andersen 1990). However, other pseudopod microtubules emanate from the nuclear envelope (O'Kelly and Wujek 1995), suggesting a different type of particle uptake. The nutritional habits of chrysomonads are varied and complex, and further study is required. For review of history, cell structure, and cellular mechanisms, see Moestrup and Andersen (1991); for review of ecological implications, see Holen and Boraas (1995).

Conversely, the Synurophyceae are all phototrophic, although it seems likely (not established) that cells will take up dissolved organic matter by osmotrophy. There is no clear evidence for phagotrophy in the group, though particle ingestion has been reported by Bird and Kalff (1987) for *Catenochrysis* (= *Synura*), and Conrad (1914) described a species of *Mallomonas* in which he observed cells of *Chlorella* that apparently had been taken up in food vacuoles. Phagotrophy, if it even exists in the

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Synurophyceae, must occur by a means different than for the Chrysophyceae because the flagellar microtubular root system is fundamentally different. Some colorless species of *Mallomonas* have also been described, but the description of these has been brought strongly into question (see Preisig et al. 1991).

Cells of the Chrysophyceae vegetatively undergo longitudinal division. Mitosis and cytokinesis are known in detail only from a few species. In *Ochromonas* and *Poterioochromonas,* the nuclear envelope is completely dispersed at metaphase (Slankis and Gibbs 1972, Bouck and Brown 1973, Tippit et al. 1980), the microtubule organizing center for the spindle poles being located on the rhizoplast. In the capsoid genus *Hydrurus,* there is a more or less intact nuclear envelope with polar fenestrae during metaphase (Vesk et al. 1984). The Synurophyceae divide vegetatively in a similar manner. Mitosis begins as flagellar transformation proceeds, and the two sets of basal bodies migrate apart (Beech and Wetherbee 1990a, 1990b). The rhizoplast acts as a microtubular organizing center (MTOC) for the spindle microtubules. The nuclear envelope remains partially intact during mitosis in *Synura* and *Chrysodidymus* (Andersen 1989b, Graham et al. 1993) but breaks down completely in *Mallomonas* (Beech and Wetherbee 1990b). Cytokinesis begins as a cleavage furrow forms at the anterior end of the cell. Cleavage proceeds posteriorly and the two daughter cells are formed.

Sexual reproduction has been observed in a few species of the Chrysophyceae (see Fott 1959; Lund 1960; Kristiansen 1963; Sandgren 1981, 1983, 1986). Undifferentiated cells may act as gametes, fuse apically, and produce a short-lived binucleate, quadriflagellate planozygote that subsequently undergoes encystment to become a binucleate hypnozygote or sexual cyst (see Figure 11-1). Autogamic processes prior to cyst formation (fusion of nuclei formed by a preceding mitosis) are also known (Sandgren 1981). Germination of sexual cysts results in the formation of one, two, or four initial vegetative cells, depending on the species. The actual processes of karyogamy and meiosis have not yet been studied in any detail. For the Synurophyceae, sexual reproduction is also poorly known. It is suggested that in *Mallomonas,* two cells acting as isogamous gametes meet at their posterior ends and fuse (Wawrik 1960, 1970, 1972; Kristiansen 1961), whereas in *Synura,* isogametes fuse at their anterior ends (Sandgren and Flanagin 1986). Sandgren and Flanagin (1986) suggest *Synura* is heterothallic because mixtures of different clones produce cyst frequencies of 1%–20%, while individual clones produce cysts at 0.001% to 0.1%. Conversely, Wawrik (1972) suggests that *Mallomonas* is homothallic. Despite these studies, definitive evidence for ploidy change is absent, and meiosis has never been demonstrated.

ECOLOGY

The Chrysophyceae usually occur in the freshwater plankton or on submersed substrates, in all bodies of water from lakes to small pools to rivers (Nicholls and Wujek 2003). A few species are able to grow on soil and snow, and some are marine (estuarine and coastal waters, open oceans, sea ice).

The Synurophyceae occur widely in all types of freshwater habitats, and a few marine species are known. *Synura* can form extensive blooms under the ice and at the time of ice melt, and a few species of *Mallomonas* (*M. acaroides, M. caudata, M. crassisquama*) will bloom in the summer (Thomasson 1970, Kristiansen 1971, Nicholls and Gerrath 1985, Nicholls 1995). The possible causes for synurophyte blooms are reviewed by Nicholls (1995).

Some species are considered indicator species because they occur in waters with narrowly defined physical and chemical parameters. The silica scales and cysts of the Chrysophyceae and Synurophyceae remain as fossils in lake sediments (e.g., Battarbee et al. 1980; Smol 1980; Smol et al. 1984a, 1984b; Smol 1995; Zeeb et al. 1996).

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The siliceous cysts (see Figure 11-6) of both chrysophytes and synurophytes are often abundant in lake sediments, and these fossil cysts are used extensively for paleolimnological work (e.g., Duff et al. 1994, 1995). It is possible to make paleolimnological reconstructions of lake histories based on these indicator species. The paleolimnological reconstructions are used to hypothesize climatic change, productivity, and pH shifts, especially with regard to lake acidification (see Smol 1995).

The biogeography and distribution of all microalgae are controversial topics. Some workers, led by Bland Finlay, argue that for organisms smaller than 1 mm, everything is everywhere (e.g., Finlay and Clarke 1999, Finlay 2002, Finlay et al. 2002). Historically, scientists believed that microorganisms were distributed by air currents as well as by waterfowl and other aquatic organisms (e.g., Messikommer 1943, Schlichting 1961, Brown et al. 1964, Schlichting and Milliger 1969, Smith 1973). These papers provide support for the distributional aspect of the Finlay hypothesis, or neutral dispersal model. Finlay's hypothesis has a second component as well, that is, living cells of all microorganisms are present in all bodies of water but often at cell levels too low to be detected. The second aspect is essentially impossible to test. If cell numbers are usually too low for detection, then (a) are they really present at extremely low (undetectable) numbers, or (b) are they absent and Finlay is wrong?

Some recent evidence suggests that at least some microalgae have restricted geographic distributions. For example, hundreds of papers have been published on silica-scaled Chrysophyceae and Synurophyceae (e.g., see Asmund and Kristiansen 1986). From these studies, some species apparently have limited geographic distributions (Kristiansen 2001a, 2001b; Vigna and Siver 2003; Kristiansen and Lind 2005; Rezácová and Neustupa 2007). For example, the silica-scaled synurophyte *T. volvocina* is known only from Australia despite the presence of robust scales that are easily identified (Pipes et al. 1991, Pipes and Leedale 1992). Similarly, for the related silica-scaled *Mallomonas*, Kristiansen (2001a) summarizes the biogeography of *Mallomonas* and shows that of 163 described species, 22 are endemic to a single continent. For example, *Mallomonas marsupialis* is known only from Australia (Croome et al. 1998), and *M. dickii* is known only from North America (Nicholls 1982). Thus, are the "missing species" too rare to be collected by routine sampling? Or, by using sampling methods that routinely collect the organisms in other bodies of water, can it be concluded that they are completely absent and that at least some species have limited geographic distributions?

Toxin Production

There is a single report of ichthyotoxicity for *Uroglena/Uroglenopsis* (Kamiya et al. 1979). The paper, written in Japanese, has received little attention in the English-speaking world.

Taste and Odor

Both the Chrysophyceae and Synurophyceae have species that cause taste and odor problems. There are a number of reviews that summarize these results (e.g., Hayes and Burch 1989; Rashash et al. 1995; Nicholls 1995; Wee et al. 1994; Hargesheimer and Watson 1996; Watson et al. 1996, 1999; Jüttner 2001; Watson et al. 2001a, 2001b; Watson 2003; Watson and Satchwill 2003; Caldwell et al. 2004; Paterson et al. 2004; Watson 2004; Satchwill et al. 2007; Watson et al. 2007).

Among the Chrysophyceae, three genera have often caused taste and odor problems: *Chrysosphaerella, Dinobryon,* and *Uroglena/Uroglenopsis*. *Chrysosphaerella longispina* and *C. brevispina* have a widespread occurrence in freshwater lakes, reservoirs, ponds, and so forth, where cell numbers may be classified as abundant to

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bloom conditions. These blooms cause "fishy" taste and odor problems (Nicholls 1980, Starmach 1985, Dürrschmidt and Croome 1985, Kristiansen and Tong 1989, Siver 1993). *Dinobryon* species also occur widely and are abundant to bloom-forming. There are reports of volatile odorous substances (Jüttner et al. 1986). *Uroglena* and *Uroglenopsis* are two similar colonial organisms that are distinguished by some workers (Lemmermann 1899, Wujek 1976) but combined as a single genus by others (Bourrelly 1968/1981; Starmach 1985). Recent molecular studies show that they are distinct from each other (Andersen 2007). Because of taxonomic confusion in the past, it is unclear whether taste and odor problems are associated with one or both of the genera. It may be more likely that *Uroglenopsis* causes the problems because it is much more widespread. A disagreeable "fishy" odor is caused by some species (Yano et al. 1988). Odor-causing problems are also known for *Poterioochromonas malhamensis* (Jüttner and Hahne 1981).

The Synurophyceae, especially *Synura,* have been implicated as a source of taste and odor for many years (Guseva 1935, Taft 1965, Collins and Kalnins 1972, Nicholls and Gerrath 1985, Nicholls 1995). Evidence suggests that *S. petersenii* is the most common source and that it imparts a fishy, melon, or cucumber odor to drinking water (Collins and Kalnins 1972, Nicholls and Gerrath 1985, Hayes and Burch 1989, Wee et al. 1994, Nicholls 1995). Several compounds are known or suspected to cause taste and odor, including oct-1-en-3-ol; pentanone-3; penten-3-one; ocatanone-3; (E,E)-2,4-heptadienal; (E,Z)-2,4-heptadienal; *trans,cis*-2,4-decadienal; *trans*-2, *cis*-6-nonadienal; ß-cyclocitral; etc. (see Hayes and Burch 1989, Wee et al. 1994, Nicholls 1995). Jüttner (1981) also reports odor problems caused by *Synura uvella.*

REFERENCES

Aaronson, S. 1993. The ultrastructure and function of the contractile vacuole. In *Ultrastructure of microalgae.* T. Berner, ed. Boca Raton, Fla.: CRC Press.

Aaronson, S., and U. Behrens. 1974. Ultrastructure of an unusual contractile vacuole in several chrysomonad phytoflagellates. *Jour. Cell Sci.*, 14:1–9.

Alberte, R.S., and R.A. Andersen. 1986. Antheraxanthin, a light-harvesting carotenoid found in a chromophyte alga. *Plant Physiol.*, 80:583–587.

Andersen, R.A. 1982. A light and electron microscopical investigation of *Ochromonas sphaerocystis* Matvienko (Chrysophyceae): The statospore, vegetative cell and its peripheral vesicles. *Phycologia,* 21:390–398.

Andersen, R.A. 1985. The flagellar apparatus of the golden alga *Synura uvella*: Four absolute orientations. *Protoplasma,* 128:94–106.

Andersen, R.A. 1987. Synurophyceae *classis nov.*, a new class of algae. *Am. Jour. Bot.*, 74:337–353.

Andersen, R.A. 1989a. Absolute orientation of the flagellar apparatus of *Hibberdia magna* comb. nov. (Chrysophyceae). *Nord. Jour. Bot.*, 8:653–669.

Andersen, R.A. 1989b. The Synurophyceae and their relationship to other golden algae. *Nova Hedwigia Beih.*, 95:1–26.

Andersen, R.A. 1990. The three-dimensional stucture of the flagellar apparatus of *Chrysosphaerella brevispina* (Chrysophyceae) as viewed by high voltage electron microscopy stereo pairs. *Phycologia,* 29:86–97.

Andersen, R.A. 1991. The cytoskeleton of chromophyte algae. *Protoplasma,* 164:143–159.

Andersen, R.A. 2007. Molecular Systematics of the Chrysophyceae and Synurophyceae. *In Unraveling the algae: The past, present and future of algal systematics.* J. Lewis and J. Brodie, eds. Boca Raton, Fla.: Taylor and Francis.

Andersen, R.A., and T.J. Mulkey. 1983. The occurrence of chlorophylls c_1 and c_2 in the Chrysophyceae. *Jour. Phycol.*, 19:289–294.

Andersen, R.A., and H. Preisig. 2002. Synurophyceae. In *An illustrated guide to the protozoa.* 2nd ed., Vol. 2. J.J. Lee, G.F. Leedale, and P.C. Bradbury, eds. Lawrence, Kan.: Society of Protozoologists.

Andersen, R.A., and R. Wetherbee. 1992. Microtubules of the flagellar apparatus

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are active during prey capture in the chrysophycean alga *Epipyxis pulchra*. *Protoplasma*, 166:8–20.

Andersen, R.A., G.W. Saunders, M.P. Paskind, and J.P. Sexton. 1993. Ultrastructure and 18S rRNA gene sequence for *Pelagomonas calceolata* gen. et sp. nov. and the description of a new algal class, the Pelagophyceae *classis nov*. *Jour. Phycol.*, 29:701–715.

Andersen, R.A., Y. van de Peer, D. Potter, J.P. Sexton, M. Kawachi, and T. LaJeunesse. 1999. Phylogenetic analysis of the SSU rRNA from members of the Chrysophyceae. *Protist*, 150:71–84.

Archibald, A.R., W.L. Cunningham, D.J. Manners, and J.R. Stark. 1963. Studies on the metabolism of Protozoa. 10. The molecular structure of reserve polysaccharides from *Ochromonas malhamensis* and *Peranema trichophorum*. *Biochem. Jour.*, 88:444–451.

Ariztia, E.V., R.A. Andersen, and M.L. Sogin. 1991. A new phylogeny for chromophyte algae using 16S-like rRNA sequences from *Mallomonas papillosa* (Synurophyceae) and *Tribonema aequale* (Xanthophyceae). *Jour. Phycol.*, 27:428–436.

Asmund, B. 1968. Studies on Chrysophyceae from some ponds and lakes in Alaska. VI. Occurrence of *Synura* species. *Hydrobiologia,* 31:497–515.

Asmund, B., and J. Kristiansen. 1986. The genus *Mallomonas* (Chrysophyceae). *Opera Botan.*, 85:1–128.

Bailey, J.C., R.R. Bidigare, S.J. Christensen, and R.A. Andersen. 1998. Phaeothamniophyceae *classis nova*: A new lineage of chromophytes based upon photosynthetic pigments, *rbcL* sequence analysis and ultrastructure. *Protist*, 149:245–263.

Battarbee, R.W., G. Cronberg, and S. Lowry. 1980. Observations on the occurrence of scales and bristles of *Mallomonas* spp. (Chrysophyceae) in the micro-laminated sediments of a small lake in Finnish North Karelia. *Hydrobiologia*, 71:225–232.

Beattie, A., E.L. Hirst, and E. Percival. 1961. Comparative structural investigations on leucosin (chrysolaminarin) separated from diatoms and laminarin from the brown algae. *Biochem. Jour.*, 79:531–537.

Beech, P.L., and R. Wetherbee. 1990a. Direct observations on flagellar transformation in *Mallomonas splendens* (Synurophyceae). *Jour. Phycol.*, 26:90–95.

Beech, P.L., and R. Wetherbee, R. 1990b. The flagellar apparatus of *Mallomonas splendens* (Synurophyceae) at interphase and its development during the cell cycle. *Jour. Phycol.*, 26:95–111.

Beech, P.L., R. Wetherbee, and J.D. Pickett-Heaps. 1990. Secretion and deployment of bristles in *Mallomonas splendens* (Synurophyceae). *Jour. Phycol.*, 26:112–122.

Bhattacharya, D., L. Medlin, P.O. Wainright, E.V. Ariztia, C. Bibeau, S.K. Stickel, and M.L. Sogin. 1992. Algae containing chlorophylls *a* and *c* are paraphyletic: Molecular evolutionary analysis of the Chromophyta. *Evolution*, 46:1801–1817.

Bird, D.F., and J. Kalff. 1987. Algal phagotrophy: regulating factors and importance relative to photosynthesis in *Dinobryon* (Chrysophyceae). *Limnol. Oceanogr.*, 32:277–284.

Bjørnland, T., and S. Liaaen-Jensen. 1989. Distribution patterns of carotenoids in relation to chromophyte phylogeny and systematics. In *The chromophyte algae: Problems and perspectives*. Systematics Association Special Vol. 38. J.C. Green, B.S.C. Leadbeater, and W.L. Diver, eds. Oxford: Clarendon Press.

Bouck, G.B. 1972. Architecture and assembly of mastigonemes. In *Advances in cell and molecular biology*. Vol. 2. E.J. DuPraw, ed. New York: Academic Press.

Bouck, G.B., and D.L. Brown. 1973. Microtubule biogenesis and cell shape in *Ochromonas*. I. The distribution of cytoplasmic and mitotic microtubules. *Jour. Cell Biol.*, 56:340–359.

Bourrelly, P. 1957. Recherches sur les Chrysophycées. *Rev. Algol., Mém. Hors-Sér.*, 1:1–412.

Bourrelly, P. 1968/1981. *Les alges d'eau douce*. Vol. 2. Paris: Boubée. (Réimpression revue et augmentée, 1981.)

Brown, R.M., Jr., D.A. Larson, and H.E. Bold. 1964. Airborne algae: Their abundance and heterogeneity. *Science*, 143:583–585.

Brugerolle, G., and G. Bricheux. 1984. Actin microfilaments are involved in scale formation of the chrysomonad cell *Synura*. *Protoplasma*, 123:203–212.

Caldwell, G., S. Watson, and M. Bentley. 2004. How to assess toxin ingestion and postingestion partitioning in zooplankton? *Jour. Plank. Res.*, 26:1369–1377.

Cavalier-Smith, T., E.E. Chao, and M.T.E.P. Allsopp. 1995. Ribosomal RNA evidence for chloroplast loss within Heterokonta: Pedinellid relationships and a revised classification of ochristan algae. *Arch. Protistenk.*, 145:209–220.

Copyright (C) 2010 American Water Works Association All Rights Reserved

Christensen, T. 1962. Alger. In *Botanik*, Bd. 2. Systematisk Botanik Nr. 2. T.W. Böcher, M.C. Lange, and T. Sørensen, eds. Copenhagen: Munksgaard.

Coleman, A.W. 1985. Diversity of plastid DNA configuration among classes of eukaryote algae. *Jour. Phycol.*, 21:1–16.

Coleman, A.W., and L.J. Goff. 1991. DNA analysis of eukaryotic algal species. *Jour. Phycol.*, 27:463–473.

Collins, R.P., and K. Kalnins. 1972. Analysis of the free amino acids in *Synura petersenii*. *Phyton*, 29:89–94.

Conrad, W. 1914. Contribution à l'étude des Flagellates. *Arch. Protistenk.*, 34:79–94.

Croome, R., J. Kristiansen, and P.A. Tyler. 1998. A description of *Mallomonas marsupialis* nov. sp. (Synurophyceae), a new chrysophyte from Australia, with comments about the endemicity of Australian freshwater algae. *Nord. Jour. Bot.*, 18:633–639.

Doflein, F. 1916. Zuckerflagellaten. *Biologisches Zentralblatt*, 36:439–447.

Duff, K.E., B.A. Zeeb, and J.P. Smol. 1994. *Atlas of chrysophycean cysts*. Dordrecht, Netherlands: Kluwer Academic Publishers.

Duff, K.E., B.A. Zeeb, and J.P. Smol. 1995. *Atlas of chrysophycean cysts*. Dordrecht, Netherlands: Kluwer Academic Publishers.

Dunlap, J.R., P.L. Walne, and H.R. Preisig. 1987. Manganese mineralization in chrysophycean loricas. *Phycologia*, 26:394–396.

Dürrschmidt, M., and R. Croome. 1985. Mallomonadaceae from Malaysia and Australia. *Nord. Jour. Bot.*, 5:285–298.

Edvardsen, B., W. Eikrem, J.C. Green, R.A. Andersen, S.Y. Moon-van der Staay, and L.K. Medlin. 2000. Phylogenetic reconstructions of the Haptophyta inferred from 18S ribosomal DNA sequences and available morphological data. *Phycologia*, 39:19–35.

Fawley, M.W. 1989. A new form of chlorophyll *c* involved in light harvesting. *Plant Physiol.*, 91:727–732.

Finlay, B.J. 2002. Global dispersal of free-living microbial eukaryotic species. *Science*, 296:1061–1063.

Finlay, B.J., and K.J. Clarke. 1999. Ubiquitous dispersal of microbial species. *Nature*, 400:828.

Finlay, B.J., E.B. Monaghan, and S.C. Maberly. 2002. Hypothesis: The rate and scale of dispersal of freshwater diatom species is a function of their global abundance. *Protist*, 153:261–273.

Foster, K.W., and R.D. Smyth. 1980. Light antennas in phototactic algae. *Microbiol. Rev.*, 44:572–630.

Fott, B. 1959. *Algenkunde*. Jena, Germany: Gustav Fischer Verlag.

Goldstein, M., J. McLachlan, and J. Moore. 2005. Morphology and reproduction of *Synura lapponica*. *Phycologia*, 44:566–571.

Graham, L.E., J.M. Graham, and D.E. Wujek. 1993. Ultrastructure of *Chrysodidymus synuroideus* (Synurophyceae). *Jour. Phycol.*, 29:330–341.

Grassé, P.P. 1926. Contribution à l'étude des Flagellés parasites. *Arch. Zool. Exp. Gén.*, 65:345–602.

Guseva, K.A. 1935. The conditions of mass development and the physiology of nutrition of *Synura*. *Mikrobiologiya*, 4:24–43 (in Russian).

Hargesheimer, E., and S.B. Watson. 1996. Treatment options for taste and odour control: dissolved air filtration, ozonation and ground activated carbon filtration. *Wat. Res.*, 30:1423–1430.

Harris, K. 1953. A contribution to our knowledge of *Mallomonas*. *Bot. Jour. Linn. Soc.*, 55:88–102.

Hayes, K.P., and M.D. Burch. 1989. Odorous compounds associated with algal blooms in South Australian waters. *Water Res.*, 23:115–122.

Herth, W. 1979. Behaviour of the chrysoflagellate alga, *Dinobryon divergens*, during lorica formation. *Protoplasma*, 100:345–351.

Herth, W., and P. Zugenmaier. 1979. The lorica of *Dinobryon*. *Jour. Ultrastr. Res.*, 69:262–272.

Herth, W., A. Kuppel, and E. Schnepf. 1977. Chitinous fibrils in the lorica of the flagellate chrysophyte *Poteriochromonas stipitata* (syn. *Ochromonas malhamensis*). *Jour. Cell Biol.*, 73:311–321.

Hibberd, D.J. 1973. Observations on the ultrastructure of flagellar scales in the genus *Synura* (Chrysophyceae). *Arch. Mikrobiol.*, 89:291–304.

Hibberd, D.J. 1976. The ultrastructure and taxonomy of the Chrysophyceae and Prymnesiophyceae (Haptophyceae): A survey with some new observations on the ultrastructure of the Chrysophyceae. *Bot. Jour. Linn. Soc.*, 72:55–80.

Hibberd, D.J. 1978. The fine structure of *Synura sphagnicola* (Korsh.) Korsh. (Chrysophyceae). *Br. Phycol. Jour.*, 13:403–412.

Holen, D.A., and M.E. Boraas. 1995. Mixotrophy in chrysophytes. In *Chrysophyte algae: Ecology, phylogeny and development*. C.D. Sandgren, J.P. Smol, and J. Kristiansen, eds. Cambridge, UK: Cambridge University Press.

Horn, S., K. Ehlers, G. Fritzsch, M.C. Gil-Rodríguez, C. Wilhelm, and R. Schnetter. 2007.

Copyright (C) 2010 American Water Works Association All Rights Reserved

Synchroma grande spec. nov. (Synchromophyceae class. nov., Heterokontophyta): An amoeboid marine alga with unique plastid complexes. *Protist*, 158:277–293.

Hutner, S.H., L. Provasoli, and J. Filfus. 1953. Nutrition of some phagotrophic freshwater chrysomonads. *Ann. N.Y. Acad. Sci.*, 56:852–862.

Jeffrey, S.W. 1989. Chlorophyll *c* pigments and their distribution in the chromophyte algae. In *The chromophyte algae: Problems and perspectives*. Systematics Association Special Vol. 38. J.C. Green, B.S.C. Leadbeater, and W.L. Diver, eds. Oxford: Clarendon Press.

Jüttner F. 1981. Detection of lipid degradation products in the water of a reservoir during a bloom of *Synura uvella*. *Appl. Environ. Microbiol.*, 41:100–106.

Jüttner, F. 2001. Liberation of 5,8,11,14,17-eicosapentaenoic acid and other polyunsaturated fatty acids from lipids as a grazer defense reaction in epilithic diatom biofilms. *Jour. Phycol.*, 37:744–755.

Jüttner, F., and B. Hahne. 1981. Volatile excretion products of *Poterioochromonas malhamensis*: Identification and formation. *Z. Pflanzenphysiol.*, 103:403–412.

Jüttner, F., B. Höflacher, and K. Wurster. 1986. Seasonal analysis of volatile organic biogenic substances (VOBS) in freshwater phytoplankton populations dominated by *Dinobryon*, *Microcystis* and *Aphanizomenon*. *Jour. Phycol.*, 22:169–175.

Kamiya, H., K. Naka, and K. Hashimoto. 1979. Ichthyotoxicity of a flagellate *Uroglena volvox*. *Bull. Jpn. Soc. Sci. Fish.*, 45:129.

Kimura, B., and Y. Ishida. 1986. Possible phagotrophic feeding of bacteria in a freshwater red tide Chrysophyceae *Uroglena americana*. *Bull. Jpn. Soc. Sci. Fish.*, 52:697–701.

Klebs, G. 1893. Flagellatenstudien. II. *Z. wiss. Zool.*, 55:353–445.

Korshikov, A.A. 1929. Studies on the Chrysomonads. I. *Arch. Protistenk.*, 67:253–290.

Kreimer, G. 1994. Cell biology of phototaxis in flagellate algae. *Int. Rev. Cytol.*, 148:229–310.

Kristiansen, J. 1961. Sexual reproduction in *Mallomonas caudata*. *Bot. Tidsskr.*, 57:306–309.

Kristiansen, J. 1963. Sexual and asexual reproduction in *Kephyrion* and *Stenocalyx* (Chrysophyceae). *Bot. Tidsskr.*, 59:244–254.

Kristiansen, J. 1971. A *Mallomonas* bloom in a Bulgarian mountain lake. *Nova Hedwigia*, 21: 877–882.

Kristiansen, J. 2001a. Biogeography of silica-scaled chrysophytes. *Nova Hedwigia Beih.*, 122:23–39.

Kristiansen, J. 2001b. Cosmopolitan chrysophytes. *Syst. Geogr. Pl.*, 70:291–300.

Kristiansen, J., and J.F. Lind. 2005. Endemicity in silica-scaled chrysophytes. *Nova Hedwigia Beih.*, 128:65–83.

Kristiansen, J., and H.R. Preisig. 2007. Chrysophyte and haptophyte algae. Teil 2, Part 2: Synurophyceae. In *Süßwasserflora von Mitteleuropa [Freshwater Flora of Central Europe]*. B. Büdel, G. Gärtner, L. Krienitz, H. Preisig, and M. Schagerl, eds. Berlin: Spektrum, Akademischer Verlag.

Kristiansen, J., and D. Tong. 1989. *Chrysosphaerella annulata* n. sp., a new scale-bearing chrysophyte. *Nord. Jour. Bot.*, 9:329–332.

Kurata, A. 1986. Blooms of *Uroglena americana* in relation to concentrations of B group vitamins. In *Chrysophytes: Aspects and problems*. J. Kristiansen and R.A. Andersen, eds. Cambridge, UK: Cambridge University Press.

Lavau, S., and R. Wetherbee. 1994. Structure and development of the scale case of *Mallomonas adamas* (Synurophyceae). *Protoplasma,* 181:259–268.

Lavau, S., G.W. Saunders, and R. Wetherbee. 1997. A phylogenetic analysis of the Synurophyceae using molecular data and scale case morphology. *Jour. Phycol.*, 33:135–151.

Leadbeater, B.S.C. 1989. The phylogenetic significance of flagellar hairs in the Chromophyta. In *The chromophyte algae: Aspects and problems*. Systematics Association Special Vol. 38. J.C. Green, B.S.C. Leadbeater, and W.L. Diver, eds. Oxford: Clarendon Press.

Leadbeater, B.S.C., and D.A.N. Barker. 1995. Biomineralization and scale production in the Chrysophyta. In *Chrysophyte algae: Ecology, phylogeny and development*. C.D. Sandgren, J.P. Smol, and J. Kristiansen, eds. Cambridge, UK: Cambridge University Press.

Leipe, D.D., P.O. Wainright, J.H. Gunderson, D. Porter, D.J. Patterson, F. Valois, S. Himmerich, and M.L. Sogin. 1994. The stramenopiles from a molecular perspective: 16S-like rRNA sequences from *Labyrinthuloides minuta* and *Cafeteria roenbergensis*. *Phycologia*, 33:369–377.

Lemmermann, E. 1899. Das Phytoplankton sächsischer Teiche. *Forschungsber. Biol. Stat. Plön.*, 7:96–135.

Ludwig, M., J.L. Lind, E.A. Miller, and R. Wetherbee. 1996. High molecular mass glycoproteins associated with the siliceous

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scales and bristles of *Mallomonas splendens* (Synurophyceae) may be involved in cell surface development and maintenance. *Planta*, 199:219–228.

Lund, J.W.G. 1960. New or rare British Chrysophyceae. 3. New records and observations on sexuality and classification. *New Phytol.*, 59:349–360.

Messikommer, E. 1943. Untersuchungen über die passive Verbreitung der Algen. *Z. Hydrol.*, 9:310–316.

Mignot, J.P., and B. Brugerolle. 1982. Scale formation in chrysomonad flagellates. *Jour. Ultrastruct. Res.*, 81:13–26.

Moestrup, Ø. 1995. Current status of chrysophyte "splinter groups": Synurophyceae, pedinellids, silicoflagellates. In *Chrysophyte algae: Ecology, phylogeny and development.* C.D. Sandgren, J.P. Smol, and J. Kristiansen. eds. Cambridge, UK: Cambridge University Press.

Moestrup, Ø., and R.A. Andersen. 1991. Organization of heterotrophic heterokonts. In *The biology of free-living heterotrophic flagellates.* Systematics Association Special Vol. 45. D.J. Patterson and J. Larsen, eds. Oxford: Clarendon Press.

Nicholls, K.H. 1980. A reassessment of *Chrysosphaerella longispina* and *C. multispina*, and a revised key to related genera in the Synuraceae (Chrysophyceae). *Pl. Syst. Evol.*, 135:95–106.

Nicholls, K.H. 1982. *Mallomonas* species (Chrysophyceae) from Ontario, Canada including descriptions of two new species. *Nova Hedwigia,* 36:89–124.

Nicholls, K.H. 1995. Chrysophyte blooms in the plankton and neuston of marine and freshwater systems. In *Chrysophyte algae: Ecology, phylogeny and development.* C.D. Sandgren, J.P. Smol, and J. Kristiansen, eds. Cambridge, UK: Cambridge University Press.

Nicholls, K.H., and J.F. Gerrath. 1985. The taxonomy of *Synura* (Chrysophyceae) in Ontario with special reference to taste and odour in water supplies. *Can. Jour. Bot.*, 63:1482–1493.

Nicholls, K.H., and D.E. Wujek. 2003. Chrysophycean algae. In *Freshwater algae of North America: Ecology and classification.* J.D. Wehr and R.G. Sheath, eds. San Diego: Academic Press.

O'Kelly, J.C., and D.E. Wujek. 1995. Status of the Chrysamoebales (Chrysophyceae): Observations on *Chrysamoeba pyrenoidifera*, *Rhizochromulina marina* and *Lagynion delicatulum*. In *Chrysophyte algae: Ecology, phylogeny and development.* C.D. Sandgren, J.P. Smol, and J. Kristiansen, eds. Cambridge, UK: Cambridge University Press.

Parke, M., I. Manton, and B. Clarke. 1955. Studies on marine flagellates. II. Three new species of *Chrysochromulina. Jour. Mar. Biolog. Assoc. UK*, 34:579–604.

Pascher, A. 1912. Über Rhizopoden und Palmellastadien bei Flagellaten (Chrysomonaden), nebst einer Übersicht über die braunen Flagellaten. *Arch. Protistenk.*, 25:153–200.

Pascher, A. 1914. Über Flagellaten und Algen. *Berichte der Deutschen botanischen Gesellschaft* 32:136–60.

Paterson, A., B. Cumming, J. Smol, and R. Hall. 2004. Marked recent increases of colonial scaled Chrysophytes in boreal lakes: Implications for the management of taste and odour events. *Freshwater Biol.*, 49:199–207.

Peters, M.C., and R.A. Andersen. 1993a. The fine structure and scale formation of *Chrysolepidomonas dendrolepidota* gen. et sp. nov. (Chrysolepidomonadaceae fam. nov., Chrysophyceae). *Jour. Phycol.*, 29:469–475.

Peters, M.C., and R.A. Andersen. 1993b. The flagellar apparatus of *Chrysolepidomonas dendrolepidota* (Chrysophyceae), a single-celled monad covered with organic scales. *Jour. Phycol.*, 29:476–485.

Petersen, J.-B., and J.B. Hansen. 1956. On the scales of some *Synura* species. *Biol. Medd. Dan. Vid. Selsk.*, 23(2):3–27.

Pipes, L.D., and G.F. Leedale. 1992. Scale formation in *Tessellaria volvocina* (Synurophyceae). *Br. Phycol. Jour.*, 27:1–29.

Pipes, L.D., G.F. Leedale, and P.A. Tyler. 1991. The ultrastructure of *Tessellaria volvocina* (Synurophyceae). *Br. Phycol. Jour.*, 26:259–278.

Preisig, H.R. 1986. Biomineralization in the Chrysophyceae. In *Biomineralization in lower plants and animals.* Systematics Association Special Vol. 30. B.S.C. Leadbeater and R. Riding, eds. Oxford: Clarendon Press.

Preisig, H.R. 1989. The flagellar base ultrastructure and phylogeny of chromophytes. In *The chromophyte algae: Problems and perspectives.* Systematics Association Special Vol. 38. J.C. Green, B.S.C. Leadbeater, and W.L. Diver, eds. Oxford: Clarendon Press.

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Preisig, H.R. 1994. Siliceous structures and silicification in flagellated protists. *Protoplasma*, 181:29–42.

Preisig, H.R., and R.A. Andersen. 2002. Chrysophyceae. In *An illustrated guide to the protozoa.* 2nd ed., Vol. 2. J.J. Lee, G.F. Leedale, and P.C. Bradbury, eds. Lawrence, Kan.: Society of Protozoologists.

Preisig, H.R., and D.J. Hibberd. 1982a. Ultrastructure and taxonomy of *Paraphysomonas* (Chrysophyceae) and related genera (1). *Nord. Jour. Bot.*, 2:397–420.

Preisig, H.R., and D.J. Hibberd. 1982b. Ultrastructure and taxonomy of *Paraphysomonas* (Chrysophyceae) and related genera (2). *Nord. Jour. Bot.*, 2:601–638.

Preisig, H.R., and D.J. Hibberd. 1983. Ultrastructure and taxonomy of *Paraphysomonas* (Chrysophyceae) and related genera (3). *Nord. Jour. Bot.*, 3:695–723.

Preisig, H.R., N. Vørs, and G. Hällfors. 1991. Diversity of heterotrophic heterokont flagellates. In *The biology of free-living heterotrophic flagellates.* Systematics Association Special Vol. 45. D.J. Patterson and J. Larsen, eds. Oxford: Clarendon Press.

Pringsheim, E.G. 1935. Über Azetatflagellaten. *Natur-wissenschaften*, 23:110–114.

Pringsheim, E.G. 1952. On the nutrition of *Ochromonas. Q. Jour. Microsc. Sci.*, 93:71–96.

Quillet, M. 1955. Sur la nature chimique de la leucosine, polysaccharide de réserve caractéristique des Chrysophycées, extraite d'*Hydrurus foetidus. C.r.heb. Séanc. Acad. Sci. Paris*, 240:1001–1003.

Rashash, D.M.C., A.M. Dietrich, R.C. Hoehn, and B.C. Parker. 1995. The influence of growth conditions on odor-compound production by two chrysophytes and two cyanobacteria. *Water Sci. Technol.*, 31:165–172.

Rezácová, M., and J. Neustupa. 2007. Distribution of the genus *Mallomonas* (Synurophyceae)—Ubiquitous dispersal in microorganisms evaluated. *Protist*, 158:29–37.

Sandgren, C.D. 1980a. An ultrastructural investigation of resting cyst formation in *Dinobryon cylindricum* Imhof (Chrysophyceae, Chrysophycota). *Protistologica*, 16:259–276.

Sandgren, C.D. 1980b. Resting cyst formation in selected chrysophyte flagellates: An ultrastructural survey including a proposal for the phylogenetic significance of interspecific variations in the encystment process. *Protistologica*, 16:289–303.

Sandgren, C.D. 1981. Characteristics of sexual and asexual resting cyst (statospore) formation in *Dinobryon cylindricum* Imhof (Chrysophyta). *Jour. Phycol.*, 17:199–210.

Sandgren, C.D. 1983. Survival strategies of chrysophycean flagellates: Reproduction and the formation of resistant resting cysts. In *Survival strategies of the algae.* G.A. Fryxell, ed. Cambridge, UK: Cambridge University Press. pp. 23-48.

Sandgren, C.D. 1986. Effects of environmental temperature on the vegetative growth and sexual life history of *Dinobryon cylindricum* Imhof. In *Chrysophytes: Aspects and problems.* J. Kristiansen and R.A. Andersen, eds. Cambridge, UK: Cambridge University Press.

Sandgren, C.D. 1989. SEM investigations of statospore (stomatocyst) development in diverse members of the Chrysophyceae and Synurophyceae. *Beih. Nova Hedwigia*, 95:45–69.

Sandgren, C.D., ed. 1991. Applications of chrysophyte stomatocysts in paleolimnology. *Jour. Paleolimnol.*, 5:1–113.

Sandgren, C.D., and S.B. Barlow. 1989. Siliceous scale production in chrysophyte algae. II. SEM observations regarding the effects of metabolic inhibitors on scale regeneration in laboratory population of scale-free *Synura petersenii* cells. *Beih. Nova Hedwigia*, 95:27–44.

Sandgren, C.D., and J. Flanagin. 1986. Heterothallic sexuality and density dependent encystment in the chrysophycean alga *Synura petersenii* Korsh. *Jour. Phycol.*, 22:206–216.

Sandgren, C.D., and W.E. Walton. 1995. The influence of zooplankton herbivory on the biogeographiy of chrysophyte algae. In *Chrysophyte algae: Ecology, phylogeny and development.* C.D. Sandgren, J.P. Smol, and J. Kristiansen, eds. Cambridge, UK: Cambridge University Press.

Sandgren, C.D., S.A. Hall, and S.B. Barlow. 1996. Siliceous scale production in chrysophyte and synurophyte algae. I. Effects of silica-limited growth on cell silica content, scale morphology, and the construction of the scale layer of *Synura petersenii. Jour. Phycol.*, 32:675–692.

Satchwill, T., S.B. Watson, and E. Dixon. 2007. Odourous algal-derived alkenes: Differences in stability and treatment responses in drinking water *Water Sci. Technol.*, 55(5):95–102.

Saunders, G.W., D. Potter, M.P. Paskind, and R.A. Andersen. 1995. Cladistic analyses of

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combined traditional and molecular data sets reveal an algal lineage. *Proc. Nat. Acad. Sci. USA*, 92:244–248.

Saunders, G.W., D.R.A. Hill, and P.A. Tyler. 1997a. Phylogenetic affinities of *Chrysonephele palustris* (Chrysophyceae) based on inferred nuclear small-subunit ribosomal RNA sequence. *Jour. Phycol.*, 33:132–134.

Saunders, G.W., D. Potter, and R.A. Andersen. 1997b. Phylogenetic affinities of the Sarcinochrysidales and Chrysomeridales based on analyses of molecular and combined data. *Jour. Phycol.*, 33:310–318.

Scherffel, A. 1901. Kleiner Beitrag zur Phylogenie einiger Gruppen niederer Organismen. *Bot. Zeit.*, 59:143–158.

Schlichting, H.E., Jr. 1961. Viable species of algae and protozoa in the atmosphere. *Lloydia*, 24:81–88.

Schlichting, H.E., Jr., and L.E. Milliger. 1969. The dispersal of microorganisms by a hemipteran, *Lethocerus uhleri* (Montandon). *Trans. Am. Microsc. Soc.*, 88:452–454.

Schnepf, E., and G. Deichgräber. 1969. Über die Feinstruktur von *Synura petersenii* unter besonderer Berücksichtigung der Morphogenese ihrer Kieselschuppen. *Protoplasma*, 68:85–106.

Schnepf, E., G. Röderer, and W. Herth. 1975. The formation of the fibrils in the lorica of *Poteriochromonas stipitata*: Tip growth, kinetics, site, orientation. *Planta (Berl.)*, 125:45–62.

Siver, P.A. 1993. Morphological and ecological characteristics of *Chrysosphaerella longispina* and *C. brevispina* (Chrysophyceae). *Nord. Jour. Bot.*, 13:343–351.

Silva, P.C. 1980. Names of classes and families of living algae. *Regnum Vegetabile*, 103:1–156.

Skuja, H. 1956. Taxonomische und biologische Studien über das Phytoplankton schwedischer Binnengewässer. *Nov. Act. Reg. Soc. Sci. Upsal.*, Ser. 4/16(3):1–404.

Slankis, T., and S.P. Gibbs. 1972. The fine structure of mitosis and cell division in the chrysophycean alga *Ochromonas danica*. *Jour. Phycol.*, 8:243–256.

Sleigh, M.A. 1964. Flagellar movement of the sessile flagellates *Actinomonas*, *Codonosiga*, *Monas*, and *Poteriodendron*. *Q. Jour. Microscop. Sci.*, 105:405–414.

Sleigh, M.A. 1974. *The biology of cilia and flagella*. London: Pergamon.

Smestad-Paulsen, B., and S. Myklestad. 1978. Structural studies of the reserve glucan produced by the marine diatom *Skeletonema costatum* (Grev.) Cleve. *Carbohydr. Res.*, 62:386–388.

Smith, P.E. 1973. The effects of some air pollutants and meteorological conditions on airborne algae and protozoa. *Jour. Air Pollut. Control Assoc.*, 23: 876–880.

Smol, J.P. 1980. Fossil synuracean (Chrysophyceae) scales in lake sediments: A new group of paleoindicators. *Can. Jour. Bot.*, 58:458–465.

Smol, J.P. 1995. Application of chrysophytes to problems in paleoecology. In *Chrysophyte algae: Ecology, phylogeny and development*. C.D. Sandgren, J.P. Smol, and J. Kristiansen, eds. Cambridge, UK: Cambridge University Press.

Smol, J.P., D.F. Charles, and D.R. Whitehead. 1984a. Mallomonadacean microfossils provide evidence of recent lake acidification. *Nature*, 307(5952):628–630.

Smol, J.P., D.F. Charles, and D.R. Whitehead. 1984b. Mallomonadacean (Chrysophyceae) assemblages and their relationships with limnological characteristics in 38 Adirondack (New York) lakes. *Can. Jour. Bot.*, 62:911–923.

Starmach, K. 1985. Chrysophyceae und Haptophyceae. In *Süsswasserflora von Mitteleuropa*. Band 1. H. Ettl, J. Gerloff, H. Heynig, and D. Mollenhauer, eds. Stuttgart: Gustav Fischer Verlag.

Taft, C.E. 1965. *Water and algae: World problems*. Chicago: Educational Publishers.

Thomasson, K. 1970. A *Mallomonas* population. *Sven. Bot. Tidskr.*, 64:303–311.

Tippit, D.H., L. Pillus, and J.D. Pickett-Heaps. 1980. Organization of spindle microtubules in *Ochromonas danica*. *Jour. Cell Biol.*, 87:531–545.

Vesk, M., L.R. Hoffman, J.D. and Pickett-Heaps. 1984. Mitosis and cell division in *Hydrurus foetidus* (Chrysophyceae). *Jour. Phycol.*, 20:461–470.

Vigna, M.S., and P.A. Siver. 2003. Biodiversity and biogeographical implications of silica-scaled chrysophytes (Chrysophyceae and Synurophyceae) of the northeast wetlands of Argentina. *Arch. Hydrobiol.*, 158:359–372.

Vlk, W. 1931. Über die Struktr der Heterokontengeisseln. *Beih. Bot. Zentralbl.*, 48:214–228.

Walne, P.L., V. Passarelli, P. Lenzi, L. Barsanti, and P. Gualtieri. 1995. Isolation of the flagellar swelling and identification of retinal in the phototactic flagellate, *Ochromonas danica* (Chrysophyceae). *Jour. Euk. Microbiol.*, 42:7–11.

Watson, S.B. 2003. Chemical communication or chemical waste? A review of the chemical ecology of algal odour. *Phycologia*, 42:333–350.

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Watson, S.B. 2004. Aquatic taste and odor: A primary signal of drinking-water integrity. *Jour. Toxicol. Environ. Health*, 67:1779–1795.

Watson, S.B., and T. Satchwill. 2003. Chrysophyte odour production: The impact of resources at the cell and population levels. *Phycologia,* 42:393–405.

Watson, S.B., E. McCauley, H. Hardisty, E. Hargesheimer, and J. Dixon. 1996. Chrysophyte blooms in oligotrophic Glenmore Reservoir (Calgary, Canada). *Nova Hedwigia*, 114:193–217.

Watson, S.B., B. Brownlee, T. Satchwill, and E. McCauley. 1999. The use of solid phase microextraction (SPME) to monitor for major organoleptic compounds produced by chrysophytes in surface waters. *Water Sci. Technol.*, 40:251–256.

Watson, S.B., T. Satchwill, and E. McCauley. 2001a. Under-ice blooms and source-water odour in a nutrient-poor reservoir: Biological, ecological and applied perspectives. *Freshwater Biol.*, 46:1–15.

Watson, S.B., T. Satchwill, and E. McCauley. 2001b. Drinking water taste and odour: A chrysophyte perspective. *Nova Hedwigia*, 122:119–146.

Watson, S.B., F. Juttner, and O. Koester. 2007. *Daphnia* behavioural responses to taste and odour compounds: Ecological significance and application as an inline treatment plant monitoring tool. *Water Sci. Technol.*, 55(5):23–31.

Wawrik, F. 1960. Sexualität bei *Mallomonas fastigata* var. *kriegerii*. *Arch. Protistenk.*, 104:541–544.

Wawrik, F. 1970. Isogamie bei *Synura petersenii* Korschikov. *Arch. Protistenk.*, 112:259–261.

Wawrik, F. 1972. Isogame Hologamie in der Gattung *Mallomonas* Perty. *Nova Hedwigia*, 23:353–362.

Wee, J.L. 1997. Scale biogenesis in synurophycean protists. *Crit. Rev. Plant Sci.*, 16:497–534.

Wee, J.L., J. Chesnick, and R.A. Cattolico. 1993. Partial characterization of the chloroplast genome from the chromophytic alga *Synura petersenii* (Synurophyceae). *Jour. Phycol.*, 29:96–99.

Wee, J.L., S.A. Harris, J.P. Smith, C.P. Dionigi, and D.F. Millie. 1994. Production of the taste/odor-causing compound *trans-2,cis-6-nonadienal*, within the Synurophyceae. *Jour. Appl. Phycol.*, 6:365–369.

Wee, J.L., J.M. Hinchey, K.X. Nguyen, P.J. Kores, and D.L. Hurley. 1996. Assessment of the phylogenetic utility of *psb*A and ITS/5.8S nucleotide sequences for the Synurophyceae. *Nova Hedwigia Beih.*, 114:29–43.

Wessel, D., and D.G. Robinson. 1979. Studies on the contractile vacuole of *Poterioochromonas malhamensis* Peterfi. I. The structure of the alveolate vesicles. *Eur. Jour. Cell Biol.*, 19:60–66.

Wetherbee, R., and R.A. Andersen. 1992. Flagella of a chrysophycean alga play an active role in prey capture and selection. Direct observations on Epipyxis pulchra using image enhanced video microscopy. *Protoplasma*, 166:1–7.

Wetherbee, R., S.J. Platt, P.L. Beech, and J.D. Pickett-Heaps. 1988. Flagellar transformation in the heterokont *Epipyxis pulchra* (Chrysophyceae): Direct observations using image-enhanced light microscopy. *Protoplasma*, 145:47–54.

Wetherbee, R., A. Koutoulis, and P.L. Beech. 1989. The role of the cytoskeleton during the assembly, secretion and deployment of scales and spines. In *Algae as experimental systems*. A.W. Coleman, L.J. Goff, and J.R. Stein-Taylor, eds. New York: A.R. Liss.

Wetherbee, R., A. Koutoulis, and R.A. Andersen. 1992. The microarchitecture of the chrysophycean cytoskeleton. In *The cytoskeleton of algae*. D. Menzel, ed. Boca Raton, Fla.: CRC Press.

Wetherbee, R., M. Ludwig, and A. Koutoulis. 1995. Immunological and ultrastructural studies of scale development and deployment in *Mallomonas* and *Apedinella*. In *Chrysophyte algae: Ecology, phylogeny and development*. C.D. Sandgren, J.P. Smol, and J. Kristiansen, eds. Cambridge, UK: Cambridge University Press.

Withers, N.W., A. Fiksdahl, R.C. Tuttle, and S. Liaaen-Jensen. 1981. Carotenoids of the Chrysophyceae. *Comp. Biochem. Physiol.*, 68B:345–349.

Wujek, D.E. 1976. Ultrastructure of flagellated chrysophytes. II. *Uroglena* and *Uroglenopsis*. *Cytologia*, 41:665–670.

Wujek, D.E., and J. Kristiansen. 1978. Observations on the bristle- and scale-production in *Mallomonas caudata* (Chrysophyceae). *Arch. Protistenk.*, 120:213–221.

Yano, H., M. Nakahara, and H. Ito. 1988. Water blooms of *Uroglena americana* and the identification of odorous compounds. *Water Sci. Technol.*, 20(8/9):75–80.

Zeeb, B., K.E. Duff, and J.P. Smol. 1996. Recent advances in the use of chrysophyte stomatocysts in paleoecological studies. *Nova Hedwigia Beih.*, 114:247–252.

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Zhang, X., I. Inouye, and M. Chihara. 1990a. An unusual short flagellum in *Mallomonas guttata* (Synurophyceae, Chrysophyta). *Bot. Mag. Tokyo*, 103(1069):97–101.

Zhang, X., I. Inouye, and M. Chihara. 1990b. Taxonomy and ultrastructure of a freshwater scaly flagellate *Mallomonas tonsurata* var. *etortisetifera* var. nov. (Synurophyceae, Chromophyta). *Phycologia*, 29:65–73.

Zimmermann, B. 1977. Flagellar and body scales in the chrysophyte *Mallomonas multiunca* Asmund. *Br. Phycol. Jour.*, 12:287–290.

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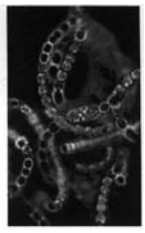

Chapter 12

Xanthophyta and Phaeophyta

John D. Wehr

BIOLOGY

Description

The algae described in this chapter are a diverse and heterogeneous collection of photosynthetic protists that vary from simple, tiny unicells—members of the so-called picoplankton such as *Nannochloropsis*—to macroscopic species that form mats in lakes, streams, and reservoirs. The most conspicuous forms are filamentous and colonize lake sediments, rocks in rapidly flowing streams, and mud banks along drainage ditches. They include well-known taxa such as the xanthophyte species *Vaucheria* and *Tribonema*, and the crust-forming phaeophyte *Heribaudiella*. At least one species is conspicuous as a symbiont in green sponges (Frost et al. 1997), while others, such as *Botrydium*, form colonies on damp soil. Many species though are microscopic, unicellular, or colonial organisms that live in planktonic or sessile aquatic habitats and are reported infrequently from few localities.

All of these organisms possess the photosynthetic pigment chlorophyll-*a*, and most also have chlorophyll-*c*, (but lack chlorophyll-*b*), plus β-carotene and various xanthophylls. The result is that many species appear pale green, yellow-green, golden, or brownish in color, rather than grass green, when viewed in a microscope. The motile cells (or motile stages) are biflagellate and heterokont (unequal), having one long tinsel flagellum (with tubular hairs) directed forward and a shorter, smooth flagellum that is directed backward. Further details of their reproduction, as well as flagellar, plastid, and general cellular ultrastructure are described elsewhere (van den Hoek et al. 1995, Graham and Wilcox 2000, Ott and Oldham-Ott 2003).

Taxonomy and Classification

The freshwater taxa considered here have traditionally been classified as members of the Xanthophyta or yellow-green algae, and in the Phaeophyta or brown algae. Current

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Table 12-1 Diagnostic characteristics of freshwater algae distinguishing the four main classes from the Xanthophyta and Phaeophyta

	Raphidophyceae	Eustigmatophyceae	Tribophyceae	Phaeophyceae
Common morphologies	Unicell, flagellate	Unicell, coccoid	Unicells Colonies Filaments	Filaments Crusts
Flagellar insertion	1 or 2, apical	1 or 2, apical	2, apical	2, lateral
Primary pigments	Chlorophyll *a*, c_1, c_2	Chlorophyll *a*	Chlorophyll *a*, c_1, c_2	Chlorophyll *a*, c_1, c_2, c_3
Color of cells or thalli	Yellow-green or yellow-brown	Yellow-green	Yellow-green or green	Brown
Major accessory pigments	β-Carotene Diatoxanthin Diadinoxanthin Vaucheriaxanthin	β-Carotene Violaxanthin Vaucheriaxanthin	β-Carotene Vaucheriaxanthin Heteroxanthin Diatoxanthin Diadinoxanthin	Fucoxanthin Violaxanthin
Chloroplasts	Ellipsoid: yellow-green	Parietal or discoid: yellow-green	Discoid: green or yellow-green	Discoid, parietal, or elongate: brown
Main storage products	Chrysolaminarin?	Chrysolaminarin	Chrysolaminarin	Laminarin
Cell wall	None (naked)	Composition uncertain	Cellulose	Alginic acid cellulose polysaccharides
No. of genera*	≈ 5	≈ 8	≈ 100	6
No. of species*	≈ 10	Unknown	≈ 600	≈ 7
Common genera	*Gonyostomum* *Vacuolaria*	*Chlorobotrys* *Nannochloropsis* *Pseudocharaciopsis*	*Botridium* *Characiopsis* *Ophiocytium* *Tribonema* *Vaucheria*	*Heribaudiella* *Pleurocladia* *Ectocarpus*†

* Freshwater taxa only.
† Common in estuarine and tidal habitats, rare in freshwater.

ultrastructural and molecular data, however, indicate that species from these groups belong to a much larger division, termed the Heterokontophyta (van den Hoek et al. 1995) or Ochrophyta (Graham and Wilcox 2000), whose unifying features include chloroplasts with four surrounding membranes, thylakoids in stacks of three, heterokont flagella in motile stages, and a particular suite of photosynthetic pigments (Sheath and Wehr 2003). Classes within the division are distinguished mainly by pigments, cellular morphology, and ultrastructure (Table 12-1), although molecular data have caused some taxa to be placed in other groups, and in some cases into new groups. Interestingly, some marine taxa in the same group have different compliments of pigments and cellular forms yet are clearly united based on rDNA evidence (Graham and Wilcox 2000). Current classification schemes now place traditional xanthophyte taxa into three classes: the Tribophyceae (Xanthophyceae), Eustigmatophyceae, and Raphidophyceae (van den Hoek et al. 1995, Ott and and Oldham-Ott 2003). There are some lesser-known classes not considered here, including the Phaeothamniophyceae (Bailey et al. 1998).

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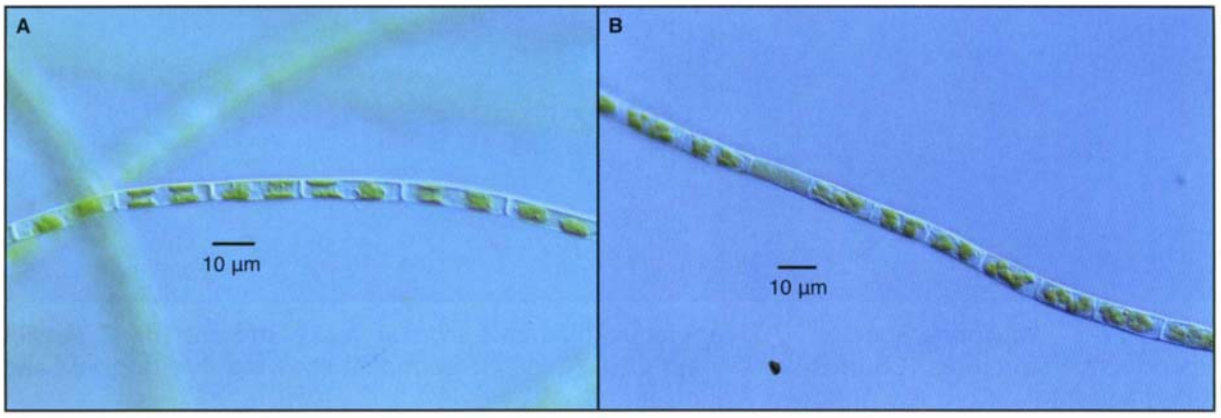

Figure 12-1 *Tribonema* species: (A) *Tribonema affine*, with two parietal chloroplasts; (B) *Tribonema vulgare*, with multiple discoid chloroplasts

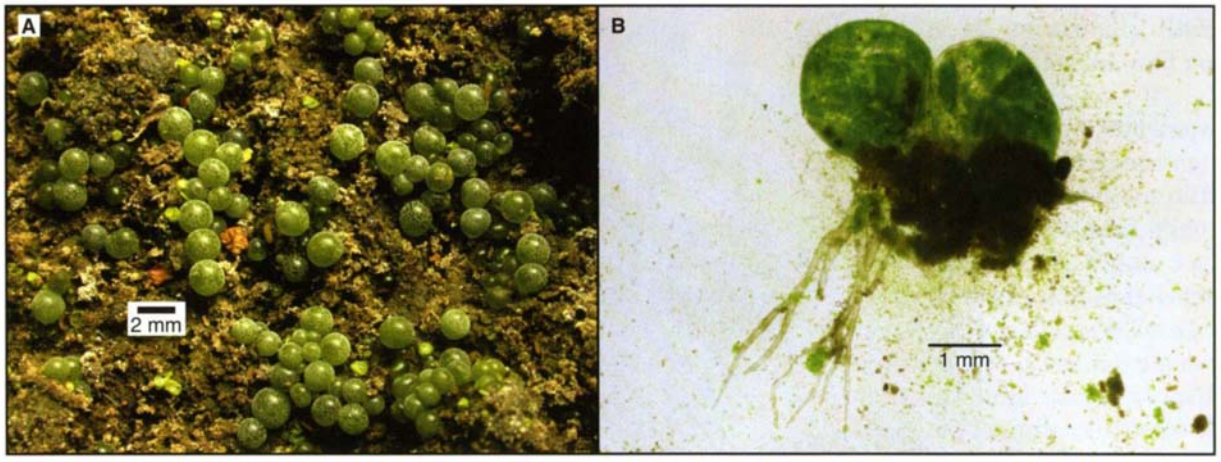

Figure 12-2 *Botrydium cf. granulatum* (A) macroscopic view of coenocytic vesicles in soil; (B) single vesicle with rhizoids (Courtesy of Malcolm Storey www.bioimages.org.uk)

Greatest freshwater diversity, in terms of species and morphology, is observed within the Tribophyceae, which include flagellates, amoeboid, palmelloid (spreading aggregations), coccoid (unicells and colonies; nonmotile; lack flagella), filamentous (unbranched and branched), and siphonous (without crosswalls) forms of cellular organization. These organisms have been classified into at least seven orders and 24 families (Ettl 1978, Ott and Oldham-Ott 2003). Some of the smaller forms, especially unicells and colonies, have morphologies that closely parallel species from other algal groups (especially in Chlorophyta and Chrysophyta), making their identification challenging (Rhodes and Stofan 1967, Ott and Oldham-Ott 2003). The presence of oil droplets and the absence of starch (using IKI stain) as a storage product usually allow individuals to distinguish these organisms from true green algae. The larger forms are simple filaments (e.g., *Tribonema*, Figure 12-1), coenocytic vesicles (e.g., *Botrydium*, Figure 12-2), or siphonous filaments (e.g., *Vaucheria*, Figure 12-3). *Vaucheria*, commonly known as "water felt," produces large and distinctive oogonia and antheridia (Figures 12-3B, 12-3C), and these structures are required for identification to species.

Members of the Raphidophyceae are flagellates, some of which are relatively large (20 to 80 µm in length) and distinguished by a long, forward-directed "hairy" flagellum (+ mastigonemes) and a finer, smooth trailing flagellum (shorter and sometimes obscure), both inserted in an apical groove termed a *gullet* (Starmach 1974, Graham

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Figure 12-3 *Vaucheria* species: (A) vegetative filaments from a rocky stream; (B) *V. sessilis* reproductive structures (Courtesy of Donald Ott); (C) *V.* cf. *geminata* showing five oogonia and curled antheridia (Courtesy of Robert Andersen)

Figure 12-4 *Pleurocladia lacustris*, showing branching filaments

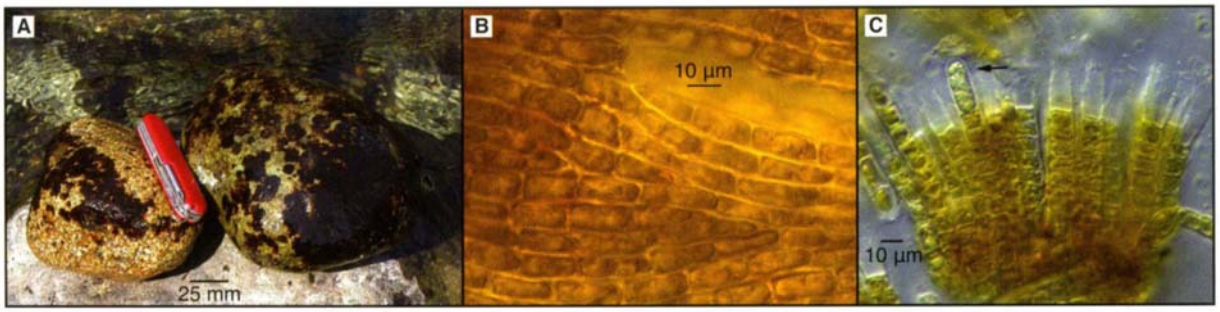

Figure 12-5 *Heribaudiella fluviatilis*: (A) macroscopic view of crusts on rocks from a creek; (B) microscopic view of prostrate (horizontal) crust of filaments; (C) microscopic view of vertical series of filaments with unilocular sporangium at arrow

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and Wilcox 2000). This arrangement causes raphidophytes to swim in short bursts. Cells often have spine- or rodlike trichocysts and a contractile vacuole. They have in past decades been classified in several different groups (e.g., Chlorophyta; Whitford and Schumacher 1984), but pigment, molecular, and ultrastructural data clearly indicate they are members of the ochrophytes (Chapman and Haxo 1966, Graham and Wilcox 2000). Cells appear yellow-greenish or brownish-green depending on the relative amounts of chlorophyll-a and xanthophylls.

Algae in the Eustigmatophyceae are represented by about eight genera (in one order; Eustigmatales) whose vegetative cells are mainly coccoid unicells or colonies (Table 12-1), and whose shape may be spherical, ovoid, or various irregular or geometric forms. Many resemble coccoid members of the Chlorophyta (Chlorococcales) but appear paler yellow-green and lack starch as a storage product. For example, commonly observed freshwater green sponges may contain either the coccoid green alga *Chlorella* (in *Spongilla lacustris*) or similar coccoid eustigmatophytes (in *Corvomeyenia everetii*) as symbionts; each are abundant within sponge tissues and critical to the sponge energy budget (Frost et al. 1997). Eustigmatophytes differ from the other xanthophytes in having only chlorophyll-*a* and violaxanthin as its primary (light-harvesting) xanthophyll. Motile cells (zooids) have either one or two flagella, and possess a distinctive orange-red eyespot, hence the name for the group.

With the exception of a few freshwater taxa, nearly all of the roughly 2,000 known species in 265 genera of brown algae (Phaeophyceae) occur in marine or estuarine environments (Wehr 2003). Freshwater species are based on a filamentous habit but differ in their gross morphology from simple (*Ectocarpus*) to branched (*Pleurocladia*, Figure 12-4), to multiaxial (*Sphacelaria*) filaments, and crustose (*Heribaudiella*, Figure 12-5) forms. They are classified as members of two or three orders (Ectocarpales, Sphacelariales, Ralfsiales), although molecular data suggest *Heribaudiella* perhaps belongs in its own order (McCauley and Wehr 2007). Pigments are similar to other members of the Ochrophyta and appear brown or golden-brown both macro- and microscopically. Cells may possess multiple discoid or ribbonlike chloroplasts, and motile cells have the two unequal flagella inserted laterally, rather than apically. Phaeophycean life cycles may have several growth forms with heteromorphic morphologies; in freshwater species these stages appear to be isomorphic. Among the freshwater species, meristematic growth can be apical (*Heribaudiella*), intercalary (*Ectocarpus*), or basal (*Pleurocladia*) (McCauley and Wehr 2007). Many species accumulate physodes in their cells, which store tannins (presumed antiherbivore function), causing collected material to become dark brown or blackish upon drying.

ECOLOGY

Occurrence and Distribution

The diversity of freshwater yellow-green algae is large, but their biology, ecology, and biogeography are known for only a few of the more common taxa. Among the Raphidophyceae, the best known species is *Gonyostomum semen* (Figure 12-6), a large flagellate often reported from dystrophic (high humic content) lakes, *Sphagnum* bogs, and other low-pH waters (Ott and Oldham-Ott 2003). This alga occasionally produces surface blooms, especially during the summer months (Le Cohu et al. 1989, Findlay et al. 2005). The aggregation of cells near the water surface in these instances is a common phenomenon, although abundances may be greater at dawn than midday (Drouet and Cohen 1935). Other species of *Gonyostomum* and *Merotrichia capitata* are reported less frequently, but apparently also occur in acidic or dystrophic environments (Whitford and Schumacher 1984). *Vacuolaria virescens* is widely reported but rarely in abundance, although Spencer (1971) observed surface blooms of this species

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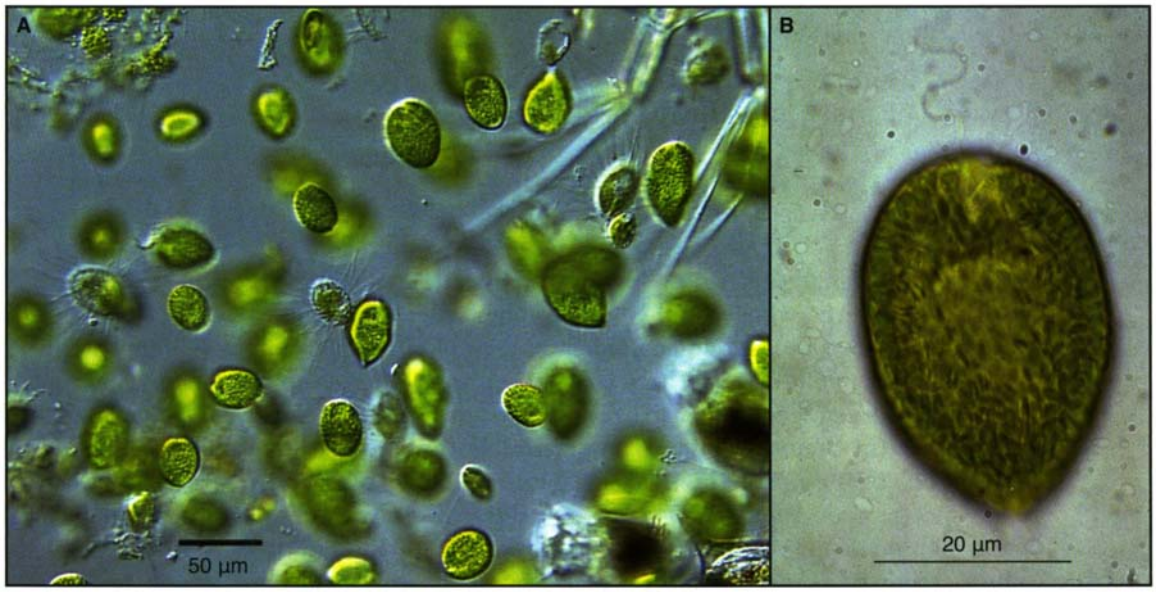

Figure 12-6 *Gonyostomum semen* (A) from a bloom collected via plankton net (Courtesy of Donald Ott); (B) close-up of a single cell and forward flagellum (Courtesy of Janet R. Stein-Taylor)

in one small circumneutral pond (pH 7.1–7.2) in early spring, just as surface ice was melting, but observed very few cells during summer. Other researchers have collected *Vacuolaria* spp. from dystrophic ponds and bogs at other times of the year (Ott and Oldham-Ott 2003). Given the apparent preference of raphidophytes and at least some eustigmatophytes to dystrophic or acidic lakes and ponds, their biogeography may be localized in boreal biomes or other regions where *Sphagnum* bogs or acidic lakes are common. Kato (1991) reported that most populations of *Gonyostomum*, *Merotrichia,* and *Vacuolaria* in Japan occurred in localities where pH levels were low. Stein and Borden (1978) report similar patterns in British Columbia.

Current knowledge of the occurrence and ecology of members of the Eustigmatophyceae is fragmentary, largely owing to difficulties and confusion in their identification. Data come mainly from general surveys and species checklists, and suggest that these organisms occur mainly in planktonic, metaphytic, and epiphytic habitats in dystrophic lakes and ponds, as well as in soils (Ott and Oldham-Ott 2003). However, few taxa have received rigorous ecological study. That these taxa have thus far been reported mainly from dystrophic and mesotrophic waters may be the result of limited sampling effort or taxonomic confusion with morphologically similar forms. *Characidiopsis ellipsoidea* was recoded an epibiont on several common crustacean zooplankton species (*Daphnia longispina* and cyclopoid copepods) in one eutrophic lake, and its abundance was inversely proportional to that of the common euglenoid epibiont *Colacium vesiculosum* (Dubovskaya et al. 2005). Given its widespread habitat, this eustigmatophyte may have a worldwide distribution. Densities up to 5.7×10^6 cells mL^{-1} have been recorded for *Nannochloropsis limnetica* in one hypereutrophic lake in Germany (Krienitz et al. 2000). It is noteworthy that this report was also a first record for this alga (i.e., a new species). An inland marine *Nannochloropsis* species (2–5 µm diameter) is reported to produce persistent blooms (mean 4.8×10^5 cells mL^{-1}) over most of the year in shallow, nutrient-poor coastal lagoons in Northern Italy (Andreoli et al. 1999). Its close resemblance to the green alga *Chlorella* or *Nannochloris* using light microscopy was resolved using high-performance liquid chromatology (HPLC) pigment analyses and 18S rRNA gene sequences. The need for such methods suggests that broader knowledge of eustigmatophyte ecology and biogeography may be slow to develop.

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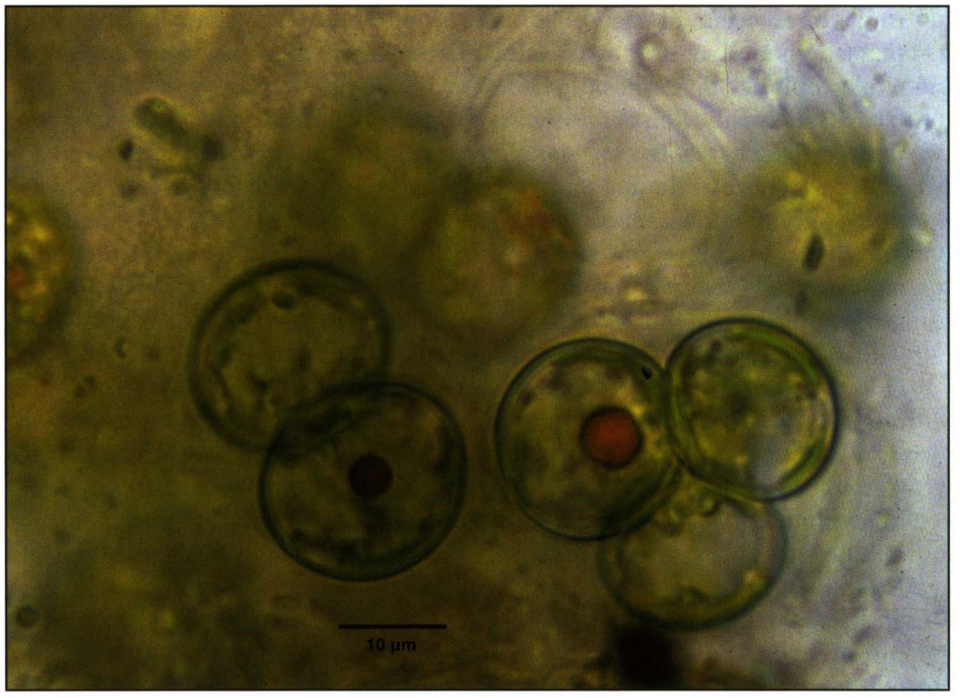

Figure 12-7 *Chlorobotrys* sp., a coccoid member of the *Eustigmatophyceae* (Courtesy of Janet R. Stein-Taylor)

More data exist on the distribution and ecology of members of the Tribophyceae, with 101 genera now reported (Ott and Oldham-Ott 2003). Knowledge is perhaps most extensive for the filamentous coenocytic genus *Vaucheria*. This benthic alga is reported from a variety of freshwater and brackish habitats worldwide, and often on sediments where it binds mud and helps retain drifting particles in lotic environments (Round 1981). There are perhaps 55 species of *Vaucheria*, most of which are regarded as cosmopolitan. Of 25 species identified from SE Australia (Entwisle 1988), 24 had been recorded from other continents, while one was named a new species. Many other reports list *Vaucheria* spp. (Stein and Borden 1978, Ott and Oldham-Ott 2003), perhaps owing to investigators not taking the steps needed to produce reproductive structures to allow species identification (Schneider et al. 1999). *Vaucheria* can be a prominent macroalga on rocks in streams (Holmes and Whitton 1977), in metaphyton of lakes and ponds, and on wetland and riparian soils (Schneider et al. 1999). On soils, propagules can be spread by earthworms (Schneider and McDevit 2002).

Species of *Tribonema* are also well represented in the freshwater algal flora (Ott and Oldham-Ott 2003) and are often observed tangled among submersed vegetation and other filamentous species. Floras list between 15 and 30 species of *Tribonema* (Starmach 1968, Ettl 1978), although regional studies typically report fewer (Prescott 1962, Whitford and Schumacher 1984). The unbranched filaments are rarely part of the true phytoplankton in freshwaters but can become dislodged and form tychoplanktonic mats on the surface of lakes. Species of this genus are reported from cool, softwater streams (Webber 1963, Sheath and Cole 1992, Vinocur and Pizarro 1995, Sheath et al. 1996, Sheath and Müller 1997), as well as in arctic and Antarctic ponds (Prescott and Vinyard 1965, Sheath and Steinman 1981). In the latter habitats, *Tribonema* may be accompanied by other xanthophytes such as *Characiopsis, Chlorobotrys* (Figure 12-7), *Ophiocytium,* and *Mischococcus.* Planktonic tribophytes, mostly unicells and small colonies, are not widely reported from riverine environments. However, *Goniochloris* spp., *Tetraedriella jovetii,* and *Pseudostaurastum lobulatum* have been

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reported to occur in greater abundances in one large river system during the limnophase, when discharge was reduced and slackwater environments enabled a buildup of phytoplankton species (de Domitrovic 2003).

The distribution of freshwater phaeophytes has been studied for nearly a century, perhaps because so few species of brown algae occur in freshwaters and due to questions about their probable marine origins (Israelson 1938, Holmes and Whitton 1975, Wehr and Stein 1985). The crustose alga *Heribaudiella fluviatilis* colonizes rocks in streams and in some wave-swept lakes (Wehr 2003). Its abundance is sufficient to be regarded as a macrophyte in some streams and rivers (Holmes and Whitton 1977). Its known geographic extent appears to be disjunct, with many populations in Europe, Japan, China, and western North America, but none known from the southern hemisphere. One other well-known taxon is the filamentous species *Pleurocladia lacustris*, which has been reported primarily from calcareous lakes and rivers, from disjunct localities in western and central Europe, western and north-central United States, and arctic Canada (Wehr 2003). A rare and possibly endemic species, *Sphacelaria lacustris*, is known globally only from one location, western Lake Michigan (Schloesser and Blum 1980). The National Oceanic and Atmospheric Administration's Great Lakes Aquatic Nonindigenous Species Information System (www.glerl.noaa.gov/res/Programs/ncrais/glansis.html), however, lists *S. lacustris* as a nonnative species. Such reports underline the need for better biogeographic and ecological studies. The lack of records of freshwater phaeophytes from certain regions may be the result of fewer studies from those areas (rather than true disjunction), although ecological conditions may prevent their presence in some regions.

Growth Conditions

Many of the freshwater species the Xanthophyta and Phaeophyta have not been isolated into unialgal culture, so that most data come from field studies. The observation that the flagellate *Gonyostomum* may aggregate near the surface of ponds, and more so at dawn than midday (Drouet and Cohen 1935), has led some to speculate that light plays a critical role in the growth or behavior of this flagellate. Findlay et al. (2005) examined possible factors triggering blooms of *G. semen* in one boreal, dystrophic lake and concluded that the blooms that developed required low irradiance (<100 μmol-photons m^{-2} sec^{-1}) and elevated total dissolved phosphorus (>30 μg L^{-1}) and DOC (>1,000 μmol L^{-1}; a cause of reduced light penetration). They also observed an inverse correlation with densities of *Daphnia rosea*, but concluded that multiple factors likely regulate *Gonyostomum* abundance. Field data (Kato 1991) and laboratory culture studies (Heywood 1973) together support the contention that growth of this and other raphidophytes is greater under low pH. Optimum growth of *G. semen* in defined media was greatest between pH 5.5 and 5.8 (with supplemental CO_2), while growth of *V. virescens* was optimized using a medium supplemented with peat extract at pH 6.0–6.2 (Heywood 1973). While blooms may be triggered by nutrient pulses, these phenomena are likely limited to moderately acidic systems.

Few data exist on factors affecting the growth of eustigmatophytes, although distribution records suggest that low pH and elevated levels of dissolved organic matter apparently favor their growth (Ott and Oldham-Ott 2003). Some species produce substantial quantities of essential fatty acids (EFAs), which in turn are required by aquatic consumers (Volkman et al. 1999). One study demonstrated that P limitation results in significant declines in EFA synthesis as well as growth rate of the eustigmatophyte *Monodus subterraneus* (Khozin-Goldberg and Cohen 2006).

Of the Tribophyceae, populations of *Vaucheria* occur over perhaps the broadest range of ecological conditions, and studies to date suggest that many individual

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species also have fairly broad tolerances. Growth and reproduction of several species of *Vaucheria* is favored in long-day (>12 hours of light) and moderate (15°–20°C) temperatures (Entwisle 1988, Schneider et al. 1999), although interspecies have been observed (Entwisle 1988, Ott and Oldham-Ott 2003). *Vaucheria* is regarded as a weed in drainage and irrigation ditches during warmer months (Fowler 1977). In such systems, *Vaucheria* growth is enhanced, along with *Cladophora, Spirogyra,* and *Oedogonium* (Chlorophyta) by elevated nutrients (esp. N and P) that prevail in these systems (Dowidar and Robson 1972, Simons 1994). Certain species may succeed in irrigation waters through tolerance to salinity (Khoja 1993). Increased nutrient inputs are blamed for the expansion of mats of *Vaucheria* spp. in Florida springs, along with the noxious cyanobacterium *Lyngbya wollei* (Pinowska et al. 2005); data suggest that a threshold of 0.6 mg total dissolved N L^{-1} promotes the development of *Vaucheria* in these springs. Entwisle (1988) reported that *Vaucheria* species, especially those colonizing riverbanks, tolerate at least short periods of emersion and desiccation.

Optimum growth of *Tribonema minus* was demonstrated to be between 15° and 25°C, and the alga outgrew *Spirogyra singularis* in mixed cultures at temperatures <15°C with low irradiance (<18 μmol-photons m^{-2} sec^{-1}; deVries and Hillebrand 1986). Field studies often record *Tribonema* populations during late winter or early spring, and in colder climates (Prescott and Vinyard 1965, Vinocur and Pizarro 1995, Sheath and Müller 1997, Nagao et al. 1999). Species such as *Tribonema ambiguum, T. australis, T. utriculosum,* and *T. viride* are regarded as typical of oligotrophic systems (Vinocur and Izaguirre 1994, Anneville et al. 2002, Bonaventura et al. 2006). Other *Tribonema* species are listed as eutrophic forms, including *Tribonema bombycinum* and various unidentified *Tribonema* species (Davis et al. 1990, Jafari and Gunale 2005). These reports collectively suggest that accurate identification is critical, if species are used as ecological indicators. Growth of a *Botrydiopsis* species isolated from the neuston (water surface) of one lake was enhanced with N limitation (low nutrients) and the presence of solid substratum (agar); its form also changed (more rhizoid growth) to more closely resemble the terrestrial alga *Botrydium* (Juarez et al. 1998). A much earlier study reported a water bloom of a *Botrydiopsis* species in one small water body (Chadefaud 1943), although more recent occurrences are not known.

SIGNIFICANCE

Source Water

No studies are known indicating that any members of the Xanthophyta or Phaeophyta are important contaminants of source water or drinking water supplies. Most studies naturally focus on eutrophic waters, with attention paid to cyanobacteria, chrysophytes, or diatoms, and occasionally green algae (Lai et al. 2002, Mhlanga et al. 2006). A lack of data does not mean that planktonic Xanthophyta do not add to the load of particulate matter or affect source water quality. Cells of some *Nannochloropsis* species (Eustigmatophyceae) likely pass through filtration systems that are less effective with other nano- and picoplanktonic-size cells (Ma et al. 2007). Scientists may overlook or misidentify some organisms as members of other groups that they resemble (Ott and Oldham-Ott 2003). For example, some species of *Centritractus, Ophiocytium*, and *Pseudotetraedon* (Tribophyceae) have needlelike morphologies that (in other species) have been reported to clog filtration systems (Jun et al. 2001). *T. bombycinum* has been listed as a filter-clogging species in past reviews (Palmer 1962), a fact that is repeated more or less verbatim in more recent government reports (Terrell and Perfetti 1989). Further careful efforts, longer seasonal studies, and the use of modern taxonomic keys are needed in water monitoring programs if data are to clarify these gaps and uncertainties.

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Treatment Plants

Few reports of algae in wastewater treatment plants mention Xanthophytes, and none have recorded any freshwater Phaeophytes. Notably, filamentous *T. bombycinum* was a common component of the periphyton at the outflow of secondary clarifying tanks of a wastewater plant in Burlington, Vt. (Davis et al. 1990). The alga was especially common during the cooler months (January–April), when other eutrophic taxa (e.g., *Oscillatoria cf. irrigua, Scenedesmus quadricauda, Stigeoclonium tenue*) were less abundant. Periphyton colonizing glass slides in alpine streams immediately downstream of sewage inputs were also dominated by *Tribonema* species along with *Stigeoclonium* sp. and *Oscillatoria* sp. (Chapman and Simmons 1990). Similarly, *Tribonema* spp. prevailed in previously nutrient-poor headwater streams during the construction phase in the creation of golf courses in north-central Ontario, Canada (Winter et al. 2003), presumably in response to elevated nutrients from fertilizers. Some studies report the mass development of water felt, *Vaucheria* spp., in eutrophic receiving wastewater nitrogen (Dowidar and Robson 1972, Simons 1994, Cole et al. 2004). In a survey of 25 New Zealand rivers, Biggs (2000) reported that the greatest biomass of benthic algae among all sites was formed by communities dominated by *Vaucheria* species in streams that received anthropogenic nutrient inputs. Water management agencies, however, should exercise caution to interpret the presence of *Vaucheria* as an indicator of wastewater or eutrophication, particularly because the alga is often reported as "*Vaucheria* spp.," based on sterile material. Palmer (1962) classified several species, including *Vaucheria geminata* and *V. sessilis,* as typical of algae attached to reservoir walls. The former species is also regarded as a weed of drainage ditches (Dowidar and Robson 1972), while the latter was also reported as abundant in pristine spring-fed streams in Ohio (Drouet 1933). The fact that some *Tribonema* and *Vaucheria* species produce substantial biomass in wastewater treatments suggests they may have promise for nutrient or heavy metal removal, as demonstrated with other filamentous species (e.g., Hoffman 1998, Pirszel et al. 1995).

Water Supply

Health concerns pertaining to algae in domestic water supplies have historically been focused on taxa known to produce toxins, or taste and odor problems (Izaguirre et al. 1982). No freshwater phaeophytes are known to be a health or water management concern in domestic water supplies. Surveys of phytoplankton communities in water supply reservoirs occasionally mention the presence of *Characidiopsis, Botrydiopsis, Characiopsis, Gonyostomum, Tribonema,* or *Vacuolaria* (e.g., Le Cohu et al. 1989, Abdul-Hussein and Mason 1988, Kuang et al. 2004), but densities are rarely great enough to be of concern. One possible solution to this problem for management agencies may be the application of pigment-discriminating cytometric methods, which have become increasingly precise in recent years. (Becker et al. 2002). A rapid and precise real-time polymerase chain reaction (PCR) method developed for detecting marine Raphidophytes (Bowers et al. 2006) may prove useful in future studies of water supplies.

Toxin Production

Freshwater xanthophyte and phaeophyte algae are not known to be toxin producers, but marine *Chattonella* and *Heterosigma* species (Raphidophyceae) produce potent ichtyotoxins that lead to massive fish kills during blooms (Marshall et al. 2004, Edvardsen and Imai 2006). These genera have no freshwater representatives, although 18s rRNA gene sequence data indicate that freshwater *V. virescens* is a sister taxon (16

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to 18 base changes) to marine raphidophytes *Chattonella subsalsa* and *Heterosigma carterae* (Potter et al. 1997).

Taste and Odor

No documented odor problems have been associated with freshwater members of the Tribophyceae, Eustigmatophyceae, Raphidophyceae, or Phaeophyceae. These classes are now placed in the Ochrophyta (Graham and Wilcox 2000), which include important odor-producing organisms in the Chrysophyceae and Synurophyceae. In 1962, Palmer listed the *Tribonema* as a source of fishy odors when abundant, but a review of the current literature did not reveal any direct links for this or any other tribophycean alga. It is difficult to know if this lack of data is the result of a true lack of odor episodes or underreporting of these algae in phytoplankton studies. It is widely recognized that lipid derivatives, especially those based on polyunsaturated fatty acids (PUFAs), are common causes of fishy odors in lakes and reservoirs (Watson 2004). This connection may prove to be important, given the very high PUFA content of several freshwater eustigmatophytes, including species of *Eustigmatos*, *Monodus*, *Nannochloropsis*, and *Vischeria* (Volkman et al. 1999, Khozin-Goldberg and Cohen 2006).

KEY FOR IDENTIFICATION TO DIVISIONS AND CLASSES

Divisions

This chapter concerns the freshwater algae traditionally (formerly) classified in the Xanthophyta and Phaeophyta; their current classification was discussed earlier.

1a. Macroscopic brown thalli, exclusively benthic with filamentous or crustose morphology; cells with golden-brown chloroplasts and fucoxanthin as the major pigment, Phaeophyta

1b. Microscopic or macroscopic greenish or yellow-green thalli, in unicells, colonies, or filaments; cells with yellow-green chloroplasts (IKI starch test negative), Xanthophyta

Classes

The only relevant class within the Phaeophyta is the Phaeophyceae (Fucophyceae), which includes all known freshwater genera. The former division Xanthophyta as considered here includes three classes: Raphidophyceae, Eustigmatophyceae, and Tribophyceae.

1a. Vegetative morphology simple; cell wall present; spherical, ovoid, or irregular-shaped, single or in colonies; prominent orange-red eyespot (outside chloroplast); plastids parietal and usually single, Eustigmatophyceae

1b. Vegetative morphology without prominent eyespot, with or without cell's wall, vegetative morphology simple or complex; unicells, colonies, or filaments, 2

2a. Unicellular, heterokont flagellates; cells typically large (30 to 80 μm long), lacking a cell wall, numerous discoid chloroplasts; trichocysts abundant, Raphidophyceae

2b. Vegetative forms diverse; cells amoeboid, flagellate, coccoid, irregular, in filaments or coenocytic forms; if flagellated, typically ≤10 μm maximum dimension; 1–3 plastids; lacking trichocysts, Tribophyceae

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USEFUL INFORMATION AND REFERENCES FOR IDENTIFICATION, WITH KEYS TO COMMON GENERA

Xanthophyta

A negative starch test is an useful first step to rule out organisms in the Chlorophyceae with similar morphologies. Fritsch (1935) and later Thomas and Goodwin (1965) reported that xanthophyll pigments (hydroxy-carotenoids) turn a blue color when treated with aqueous hydrochloric acid. Useful and more extensive references for identification of eustigmatophytes, raphidophytes, and tribophytes are provided below. Keys and advice for the more common genera are provided by class.

Eustigmatophyceae

Care is needed because nonmotile stages (the usual vegetative form) may be confused with coccoid green algae (Chlorococcales) but fail the starch test. Chloroplasts are parietal and usually single, although *Chlorobotrys* may have two or three (Figure 12-7). Useful and more extensive references for identification of eustigmatophytes include Starmach (1968), Ettl (1978), and Ott and Oldham-Ott (2003). Of the eight freshwater genera currently recognized, none can be considered common; for a complete key, refer to Ott and Oldham-Ott (2003).

Raphidophyceae

For purposes of collection and identification of these flagellates, cells placed in a clear dish or flask aggregate toward a light (Chapman and Haxo 1966). It should be noted that these organisms may produce palmelloid masses or asexual cystlike stages during specific times of the year (Drouet and Cohen 1935, Spencer 1971, Figueroa and Rengefors 2006). Authors currently recognize three freshwater genera, *Gonyostomum* (Figures 12-1A, 12-1B), *Merotrichia*, and *Vacuolaria*. Useful references for identification of raphidophytes include Starmach (1974) and Ott and Oldham-Ott (2003). They can be distinguished as follows:

1a. Spherical, ovoid, or pear-shaped cells that appear flattened; may exhibit metaboly (changes shape); chloroplasts numerous, mostly peripheral; flagella insertion apical, 2

1b. Cells not flattened, without metaboly, flagella subapical; gullet tubular, *Merotrichia*

2a. Cells ovoid or slightly pear-shaped, narrowed apically; contractile vacuole(s) prominent; gullet short or obscure, *Vacuolaria*

2b. Cells pear-shaped or ovoid, narrower at posterior end; contractile vacuole absent or not apparent; gullet appears conical (Figures 12-6A and 12-6B), *Gonyostomum*

Tribophyceae

More than 100 freshwater genera are recognized although few have been reported widely or in abundance. Some are very delicate and may be lost during preservation (e.g., Lugol's iodine); fresh samples should be examined prior to preservation and enumeration. Useful references include Blum (1972), Ettl (1978), Rieth (1980), Entwisle (1988), Schneider et al. (1999), and Ott and Oldham-Ott (2003). Of the many genera,

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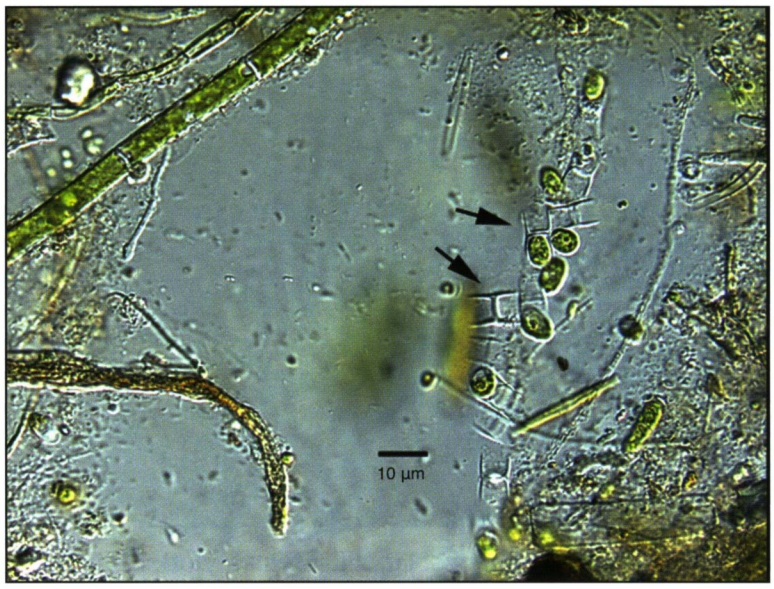

Figure 12-8 *Bumilleria* sp. showing filament and fragmented H-pieces (arrow) (Courtesy of Donald Ott)

perhaps six or seven are commonly observed in surface waters. Comprehensive keys separate the genera by order and family. The following is an artificial key to some of the common genera:

1a. Growth form filamentous, 2

1b. Nonfilamentous; unicells, aggregations, colonies, or coenocytic vesicles, 4

2a. Filaments coenocytic with few or no crosswalls (Figure 12-3A); rarely or occasionally branched; numerous yellow-green chloroplasts; produce distinct M and F reproductive structures (Figure 12-3B, 12-3C), *Vaucheria*

2b. Filaments with crosswalls and unbranched; chloroplast single or several, 3

3a. Filaments typically long; cells elongate-cylindrical; overlapping H-shaped pieces usually evident in filamentous condition (Figure 12-1A, 12-1B), *Tribonema*

3b. Filaments long or short; cells short-cylindrical or quadrate; H-shaped pieces not apparent unless fragmented (Figure 12-8), *Bumilleria*

4a. Thallus coenocytic spherical or ovoid vesicles, macroscopic when older (Figure 12-2), *Botrydium*

4b. Cells not organized as in a coenocytic vesicle; unicells or colonies, 5

5a. Spherical or ellipsoid cells grouped in twos or fours, connected by long, gelatinous threads forming branched, dendritic colonies, *Mischococcus*

5b. Cells elongate or triangular, not connected by long, gelatinous threads, 6

6a. Cells triangular or pyramidal, loosely organized in small clusters (Figure 12-9A), *Goniochloris*

6b. Cells elongate-cylindrical, curved or twisted; solitary or colonial (Figure 12-9B), *Ophiocytium*

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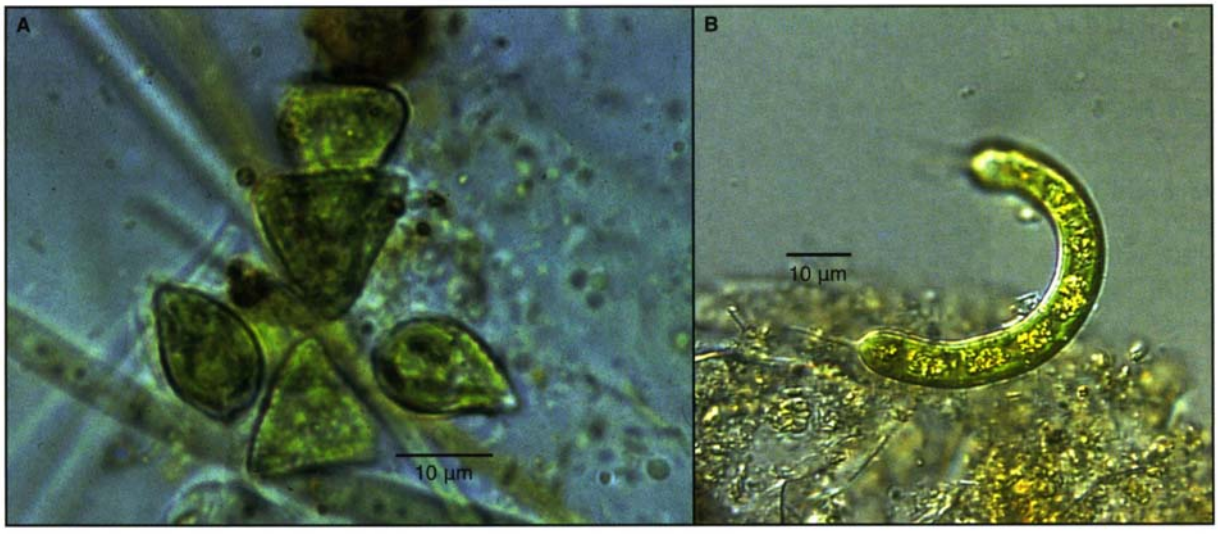

Figure 12-9 (A) *Goniochloris* sp. (Courtesy of Janet R. Stein-Taylor); (B) *Ophiocytium* cf. *capitatum* (Courtesy of Donald Ott)

Phaeophyceae

Freshwater brown algae colonize rocks in streams and large lakes. All have distinctively brown or golden-brown chloroplasts, although thalli can appear dark or light brown, depending on $CaCO_3$ precipitation (especially *Pleurocladia*). Of the six or seven known taxa, only two have been collected from several locations in the United States. Useful references include Starmach (1977) and Wehr (2003). Two common genera can be separated as follows:

1a. Thalli encrusting dark brown patches on rocks (Figure 12-5A); prostrate system of densely packed branched filaments (Figure 12-5B); vertical series of unbranched or dichotomously branched filaments (Figure 12-5C); numerous discoid brown chloroplasts, *Heribaudiella*

1b. Thalli filamentous, spreading tufts; filaments uniseriate; broadly spreading branches; basal filaments creeping on substratum or arched; erect filaments spreading; 1 (rarely 2) large parietal chloroplast(s) per cell (Figure 12-4), *Pleurocladia*

REFERENCES

Abdul-Hussein, M.M., and C.F. Mason. 1988. The phytoplankton community of a eutrophic reservoir. *Hydrobiologia*, 169:265–277.

Andreoli, C., E. Bresciani, I. Moro, L. Scarabel, N. La Rocca, L. Dalla Valle, and F. Ghion. 1999. A survey on a persistent greenish bloom in the Comacchio Lagoons (Ferrara, Italy). *Bot. Mar.*, 42:467–479.

Anneville, O., V. Ginot, and N. Angeli. 2002. Restoration of Lake Geneva: Expected versus observed responses of phytoplankton to decreases in phosphorus. *Lakes Reservoirs Res. Manage.*, 7:67–80.

Bailey, J.C., R.R. Bidigare, S.J. Christensen, and R.A. Andersen. 1998. Phaeothamniophyceae *classis nova*: A new lineage of chromophytes based upon photosynthetic pigments, *rbc*L sequence analysis and ultrastructure. *Protist*, 149:245–263.

Becker, A., A. Meister, and C. Wilhelm. 2002. Flow cytometric discrimination of various phycobilin-containing phytoplankton groups in a hypertrophic reservoir. *Cytometry*, 48:45–57.

Biggs, B.F. 2000. Eutrophication of streams and rivers: Dissolved nutrient-chlorophyll relationships for benthic algae. *Jour. N. Am. Bentholog. Soc.*, 19:17–31.

Blum, J.L. 1972. Vaucheriaceae. In *North American flora*. Series II, Part 8. New York: New York Botanical Garden.

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Bonaventura, S.M., A. Vinocur, L. Allende, and H. Pizarro. 2006. Algal structure of the littoral epilithon in lentic water bodies at Hope Bay, Antarctic Peninsula. *Polar Biol.*, 29:668–680.

Bowers, H.A., C. Tomas, T. Tengs, J.W. Kempton, A.J. Lewitus, and D.W. Oldach. 2006. Raphidophyceae (Chaedfaud ex Silva) systematics and rapid identification: Sequence analyses and real-time PCR assays. *Jour. Phycology*, 42:1333–1348.

Chadefaud, M. 1943. Su rune de "fleur d'eau" formée par des Xanthophycées du G. *Botrydiopsis* [A kind of water bloom formed by Xanthophyceae of the genus *Botrydiopsis*]. *Rev. Sci. Paris*, 81:393–396.

Chapman, D.J., and F.T. Haxo. 1966. Chloroplast pigments of the Chloromonadophyceae. *Jour. Phycol.*, 3:89–91.

Chapman, J.C., and B.L. Simmons. 1990. The effects of sewage on alpine streams in Kosciusko National Park, NSW. *Jour. Environ. Monitor. Assess.*, 14:275–295.

Cole, M.L., I. Valiela, K.D. Kroeger, G.L. Tomasky, J. Cebrian, C. Wigand, R.A. McKinney, S.P. Grady, and M.H. Carvalho da Silva. 2004. Assessment of a $\delta^{15}N$ isotopic method to indicate anthropogenic eutrophication in aquatic ecosystems. *Jour. Environ. Qual.*, 33:124–132.

Davis, L.S., J.P. Hoffmann, and P.W. Cook. 1990 Succession of algal periphyton from a wastewater treatment facility. *Jour. Phycol.*, 26:611–617.

de Domitrovic, Y.Z. 2003. Effect of fluctuations in water level on phytoplankton development in three lakes of the Parana river floodplain (Argentina). *Hydrobiologia*, 510:175–193.

deVries, P.J.R., and H. Hillebrand. 1986. Growth control of Tribonema minus and *Spirogyra singularis* by light and temperature. *Acta Bot. Neerland.*, 35:65–70.

Dowidar, A.R., and T.O. Robson. 1972. Studies on the biology and control of *Vaucheria dichotoma* found in fresh waters in Britain. *Weed Res.*, 12:221–228.

Drouet, F. 1933. Algal vegetation of the large Ozark springs. *Trans. Am. Microscop. Soc.*, 52:83–100.

Drouet, F., and A. Cohen. 1935. The morphology of *Gonyostomum semen* from Woods Hole, Massachusetts. *Biolog. Bull.*, 68:422–439.

Dubovskaya, O.P., E.P. Klimova, V.I. Kolmakov, N.A. Gaevsky, and E.A. Ivanova. 2005. Seasonal dynamic of phototrophic epibionts on crustacean zooplankton in a eutrophic reservoir with cyanobacterial bloom. *Aquat. Ecol.*, 39:167–180.

Edvardsen, B., and I. Imai. 2006. The ecology of harmful flagellates within Prymnesiophyceae and Raphidophyceae, In *Ecology of harmful algae.* E. Granéli and J.T. Turner, eds. Berlin: Springer-Verlag.

Entwisle, T.J. 1988. A monograph of *Vaucheria* (Vaucheriaceae, Chrysophyta) in southeastern mainland Australia. *Aust. System. Bot.*, 1: 1–77.

Ettl, H. 1978. Xanthophyceae. In *Süßwasserflora von Mitteleuropa.* Band 3, Teil 1. Stuttgart: Gustav Fischer Verlag.

Figueroa, R.I., and K. Rengefors. 2006. Life cycle and sexuality of the freshwater raphidophyte *Gonyostomum semen* (Raphidophyceae). *Jour. Phycol.*, 42:859–871.

Findlay, D.L., M.J. Paterson, L.L. Hendzel, and H.J. Kling. 2005. Factors influencing *Gonyostomum semen* blooms in a small boreal reservoir lake. *Hydrobiologia*, 533:243–252.

Fowler, M.C. 1977. Laboratory of a new trazine herbicide DPX-3674 on various aquatic species of macrophytes and algae. *Weed Res.*, 17:191–195.

Fritsch, F.E. 1935. *Structure and reproduction of the algae.* Vol. 1. London: Cambridge University Press.

Frost, T.M., L.E. Graham, J.E. Elias, M.J. Haase, D.W. Kretchmer, and J.A. Kranzfelder. 1997. A yellow-green algal symbiont in the freshwater sponge, *Corvomeyenia everetii*: Convergent evolution of symbiotic associations. *Freshwater Biol.*, 38:395–399.

Graham, L.D., and L.W. Wilcox. 2000. *Algae.* Upper Saddle River, N.J.: Prentice-Hall.

Heywood, P. 1973. Nutritional studies on the Chloromonadophyceae: *Vacuolaria virescens* and *Gonyostomum semen*. *Jour. Phycol.*, 9:156–159.

Hoffman, J.P. 1998. Wastewater treatment with suspended and nonsuspended algae. *Jour. Phycol.*, 34:757–763.

Holmes, N.T.H., and B.A. Whitton. 1975. Notes on some macroscopic algae new or seldom recorded for Britain: *Nostoc parmelioides, Heribaudiella fluviatilis, Cladophora aegagropila, Monostroma bullosum, Rhodoplax schinzii. Vasculum*, 60:47–55.

Holmes, N.T.H., and B.A. Whitton. 1977. The macrophytic vegetation of the River Tees in 1975: Observed and predicted changes. *Freshwater Biol.*, 7:43–60.

Israelson G. 1938. Über die Süsswasserphaeophycéen Schewedens. *Botaniska Notiser*, 1938:113–128.

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Izaguirre, G., C.J. Hwang, S.W. Krasner, and M.J. McGuire. 1982. Geosmin and 2 methylisoborneol from cyanobacteria in three water supply systems. *Appl. Environ. Microbiol.*, 43:708–714.

Jafari, N.G., and V.R. Gunale. 2005. Hydrobiological study of algae of an urban freshwater river. *Jour. Appl. Sci. Environ. Manage.*, 10:153–158.

Juarez, A.B., J.S. Alberghina, and C.G. Velez. 1998. Culture studies of *Botrydiopsis*-like morphotypes isolated from a neustonic population of *Botrydium* (Tribophyceae, Chromophyta). *Arch. für Hydrobiol. Suppl.*, 126:109–115.

Jun, H.B., Y.-J. Lee, B.D. Lee, and D.R.U. Knappe. 2001. Effectiveness of coagulants and coagulant aids for the removal of filter-clogging Synedra. *Jour. Water Supply Res. Technol.*, 50:135–148.

Kato, S. 1991. Geographic distribution of freshwater Raphidophycean algae in Japan and their effect of pH on their growth. *Jpn. Jour. Phycol.*, 39:179–184.

Khoja, T.M. 1993. Water composition and filamentous algae in the irrigation and drainage networks of Al-Hassa Oases, Saudi Arabia. *Cryptogamic Bot.*, 4:1–7.

Khozin-Goldberg, I., and Z. Cohen. 2006. The effect of phosphate starvation on the lipid and fatty acid composition of the fresh water eustigmatophyte *Monodus subterraneus*. *Phytochemistry*, 67:696–701.

Krienitz, L., D. Hepperle, H.-B. Stich, and W. Weiler. 2000. Nannochloropsis limnetica (Eustigmatophyceae), a new species of picoplankton from freshwater. *Phycologia*, 39:219–227.

Kuang, O., Y. Bi, Y. Xia, and Z. Hu. 2004. Phytoplankton community and algal growth potential in Taipinghu Reservoir, Anhui Province, China. *Lakes Reservoirs Res. Manage.*, 9:119–124.

Lai, W.-L., H.H. Yeh, I.-C. Tseng, T.-F. Lin, J.J. Chen, and G.T. Wang. 2002. Conventional versus advanced treatment for eutrophic source water. *Jour. AWWA*, 94:96–108.

Le Cohu, P.R., J. Guitard, N. Comoy, and J. Brabet. 1989. *Gonyostomum semen* a potential nuisance in large French reservoirs? The case of the Pareloup Lake. *Arch. für Hydrobiol.*, 117:225–236.

Ma, J., G. Lei, and J. Fang. 2007. Effect of algae species population structure on their removal by coagulation and filtration processes—A case study. *Jour. Water Supply Res. Technol.*, 56:41–54.

Marshall, J.A., P.D. Nichols, G.M. Hallegraeff. 2004. Ichthyotoxicity of *Chattonella marina* (Raphidophyceae) to damselfish (Acanthochromis polycanthus): The synergistic role of reactive oxygen species and free fatty acids. *Harmful Algae*, 2:273–281.

McCauley, L.A.R., and J.D. Wehr. 2007. Taxonoic reappraisal of the freshwater brown algae *Bodanella, Ectocarpus, Heribaudiella*, and *Pleurocladia* (Phaeophyceae) on the basis of *rbc*L sequences and morphological characters. *Phycologia*, 46:429–439.

Mhlanga, L., J. Day, G. Cronberg, M. Chimbari, N. Siziba, and H. Annadotter. 2006. Cyanobacteria and cyanotoxins in the source water from Lake Chivero, Harare, Zimbabwe, and the presence of cyanotoxins in drinking water. *Afr. Jour. Aquat. Sci.*, 31:165–173.

Nagao, M., K. Arakawa, D. Takezawa, S. Yoshida, S. Fujikawa. 1999. Akinete formation in *Tribonema bombycinum* Derbes et Solier (Xanthophyceae) in relation to freezing tolerance. *Jour. Plant Res.*, 112:163–174.

Ott, D.W., and C.K. Oldham-Ott. 2003. Eustigmatophyte, Raphidophyte, and Tribophyte Algae. In *Freshwater algae of North America: Ecology and classification*. J.D. Wehr and R.G. Sheath, eds. San Diego: Academic Press.

Palmer, C.M. 1962. *Algae in water supplies*. US Public Health Service Publication 657. Washington, D.C.: US Public Health Service.

Pinowska, A., R.J. Stevenson, and R. Frydenborg. 2005. Abundance of macroalgae in Florida springs: Understanding the role of nutrients. *Eos Trans. AGU*, 86(18), Joint Assembly Suppl., Abstract NB14E-06. Washington, D.C.: American Geophysical Union.

Pirszel, J., B. Pawlik, and T. Skowrofiski. 1995. Cation-exchange capacity of algae and cyanobacteria: A parameter of their metal sorption abilities. *Jour. Ind. Microbiol.*, 14:319–322.

Potter, D., G.W. Saunders, and R.A. Andersen. 1997. Phylogenetic relationships of the Raphidophyceae and Xanthophyceae as inferred from nucleotide sequences of the 18S ribosomal RNA gene. *Am. Jour. Bot.*, 84:966–972.

Prescott, G.W. 1962. *Algae of the western Great Lakes area*. Dubuque, Iowa: Wm. C. Brown.

Prescott, G.W., and W.C. Vinyard. 1965. Ecology of Alaskan freshwater algae V. Limnology and flora of Malikpuk Lake. *Trans. Am. Microscop. Soc.*, 84:427–478.

Rhodes, R.G., and P.E. Stofan. 1967. *Tetraspora, Chlorosaccus*, and *Phaeosphaera*, a unique example of parallel evolution in the algae. *Jour. Phycol.*, 3:87–89.

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Rieth, A. 1980. Xanthophyceae. In *Süßwasserflora von Mitteleuropa*. Band 4, Teil 2. Stuttgart: Gustav Fischer Verlag.

Round, F.E. 1981. *The ecology of algae*. Cambridge, UK: Cambridge University Press.

Schloesser, R.E., and J.L. Blum. 1980. *Sphacelaria lacustris* sp. nov., a freshwater brown alga from Lake Michigan. *Jour. Phycol.*, 16:201–207.

Schneider, C.W., C.E. Lane, and A. Norland. 1999. The freshwater species of *Vaucheria* (Tribophyceae, Chrysophyta) from Connecticut. *Rhodora*, 101:235–263.

Schneider, C.W., and D.C. McDevit. 2002. Are earthworms a possible mechanism for airborne dispersal of the alga *Vaucheria*? *Northeastern Nat.*, 9:225–234.

Sheath, R.G., and K.M. Cole. 1992. Biogeography of stream macroalgae in North America. *Jour. Phycol.*, 28:448–460.

Sheath, R.G., and K.M. Müller. 1997. Distribution of stream macroalgae in four high arctic drainage basins. *Arctic*, 50:355–364.

Sheath, R.G., and A.D. Steinman. 1981. A checklist of freshwater algae of the Northwest Territories, Canada. *Can. Jour. Bot.*, 60:1964–1997.

Sheath, R.G., and J.D. Wehr. 2003. Introduction to the freshwater algae. In *Freshwater algae of North America: Ecology and classification*. J.D. Wehr and R.G. Sheath, eds. San Diego: Academic Press.

Sheath, R.G., M.L. Vis, J.A. Hambrook, and K.M. Cole. 1996. Tundra stream macroalgae of North America: Composition, distribution, and physiological adaptations. *Hydrobiologia*, 336:67–82.

Simons, J. 1994. Field ecology of freshwater macroalgae in pools and ditches, with special attention to eutrophication. *Aquat. Ecol.*, 28:25–33.

Spencer, L.B. 1971. A study of *Vacuolaria virescens* Cienkowski. *Jour. Phycol.*, 7:274–279.

Starmach, K. 1968. Xanthophyceae—Różnowiciowe. *Flora slodkowodna Polski*. Vol. 7. Warsaw/Krakow: Panstwowe Wydawnictwo Naukowe.

Starmach, K. 1974. Cryptophyceae—Dinophyceae—Raphidophyceae. *Flora slodkowodna Polski*. Vol. 4. Warsaw/Krakow: Panstwowe Wydawnictwo Naukowe.

Starmach, K. 1977. Phaeophyta-brunatnice. Rhodophyta—krasnorosty. *Flora slodkowodna Polski*. Vol. 14. Warsaw/Krakow: Panstwowe Wydawnictwo Naukowe.

Stein, J.R., and C.A. Borden. 1978. A checklist of freshwater algae of British Columbia. *Syesis*, 12:3–39.

Terrell, C.R., and P.B. Perfetti. 1989. Water quality indicators guide: Surface waters. US Department of Agriculture (USDA) Soil Conservation Service, SCS-TP-161. Washington, D.C.: USDA.

Thomas, D.M., and T.W. Goodwin. 1965. Nature and distribution of carotenoids in the Xanthophyta (Heterokontae). *Jour. Phycol.*, 1:118–121.

van den Hoek, C., D.G. Mann, and H.M. Jahns. 1995. *Algae: An introduction to phycology*. Cambridge, UK: Cambridge University Press.

Vinocur, A., and I. Izaguirre. 1994. Freshwater algae (excluding Cyanophyceae) from nine lakes and pools of Hope Bay, Antarctic Peninsula. *Antarct. Sci.*, 6:483–489.

Vinocur, A., and H. Pizarro. 1995. Periphyton flora of some lotic and lentic environments of Hope Bay (Antarctic Peninsula). *Polar Biol.*, 15:401–414.

Volkman, J.K., S.M. Barrett, and S.I. Blackburn. 1999. Fatty acids and hydroxyl fatty acids in three species of freshwater Eustigmatophytes. *Jour. Phycol.*, 35:1005–1012.

Watson, S.B. 2004. Aquatic taste and odor: A primary signal of drinking-water integrity. *Jour. Toxicol. Environ. Health Part A*, 67: 1779–1795.

Webber, E.E. 1963. The ecology of some algae in Worcester County, Massachusetts. *Am. Midland Nat.*, 70:175–186.

Wehr, J.D. 2003. Brown algae. In *Freshwater algae of North America: Ecology and classification*. J.D. Wehr and R.G. Sheath, eds. San Diego: Academic Press.

Wehr, J.D., and J.R. Stein. 1985. Studies on the biogeography and ecology of the freshwater phaeophycean alga *Heribaudiella fluviatilis*. *Jour. Phycol.*, 21:81–93.

Whitford, L.A., and G.J. Schumacher. 1984. *A manual of freshwater algae*. Raleigh, N.C.: Sparks Press.

Winter, J.G., P.J. Dillon, C. Paterson, R.A. Reid, and K.M. Somers. 2003. Impacts of golf course construction and operation on headwater streams: Bioassessment using benthic algae. *Can. Jour. Bot.*, 81:848–858.

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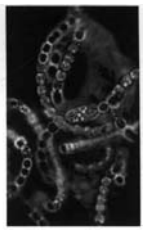

Chapter 13

Rhodophyta

Robert G. Sheath

GENERAL INTRODUCTION TO THE RED ALGAE

The Rhodophyta, the red algae, represents a phylum of organisms that share the following combination of attributes: cells with organelles (e.g., chloroplast), lack of flagella in any life history phase, storage products that are branched glucose polymers (floridean starch), blue- and red-colored pigments (phycobiliproteins), unstacked photosynthetic membranes (thylakoids), and chloroplasts with two external membranes (envelope) (Sheath and Sherwood 2002).

BIOLOGY

Freshwater red exhibit a variety of morphologies, the simplest of which are the unicells that occur as clumps on moist substrata, or pseudofilaments that are composed of a loose chain of cells in a common gelatinous matrix (Figure 13-1).

Uniseriate (single series), branched filaments may have alternate, opposite, or whorled branching (Figure 13-2). Corticated uniseriate filaments have the main axis covered with smaller photosynthetic cells that may be spherical or elongate. Multiseriate filaments consist of a central medulla of interwoven filaments from which radiate

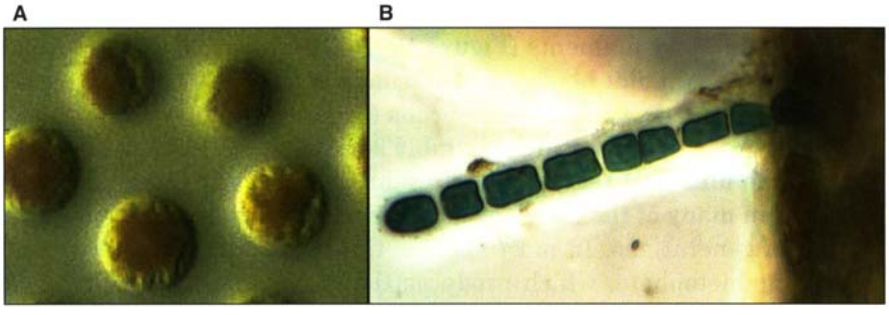

Figure 13-1 *Porphyridium purpureum*, a unicellular red alga with an axial, stellate chloroplast (A), and *Chroodactylon ornatum*, a pseudofilamentous red alga (B)

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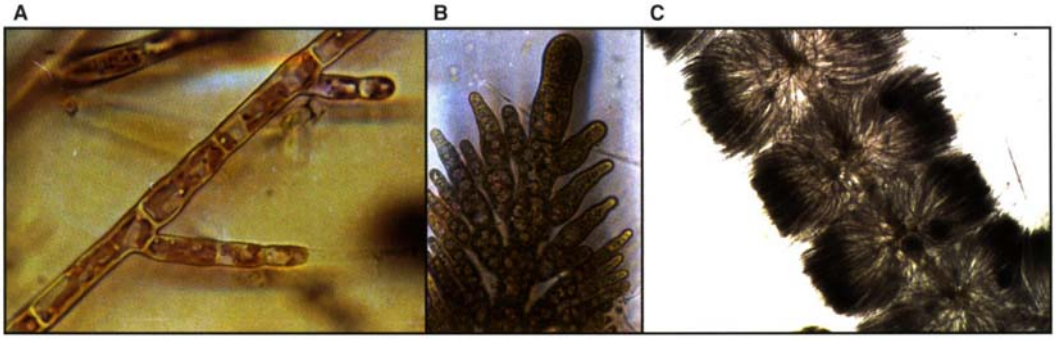

Figure 13-2 *Audouinella hermannii*, a simple, branched filament (A); *Balliopsis prieurii*, an opposite-branched filament (B); and *Batrachospermum gelatinosum*, a whorled-branched filament (C)

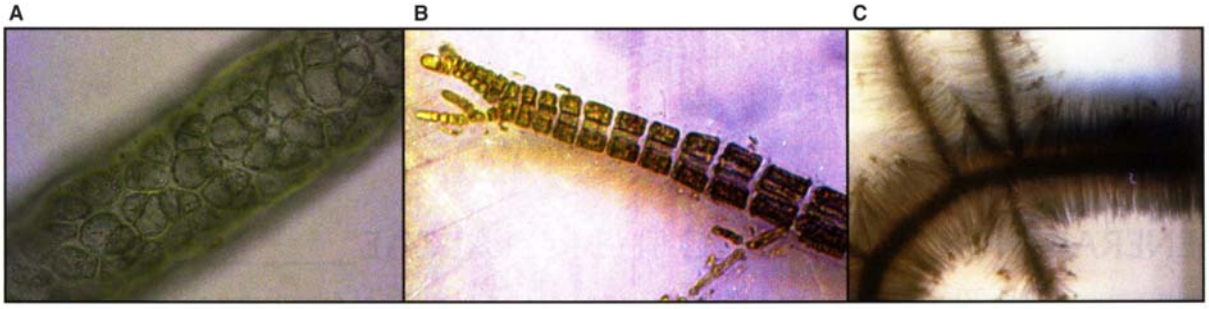

Figure 13-3 *Compsopogon coeruleus*, a uniseriate filament with an outer layer of small cortical cells (A); *Polysiphonia subtilissima*, a uniseriate filament with elongate outer pericentral cells (B); and *Thorea hispida*, a branched multiseriate filament with the medulla as a dark, inner layer (C)

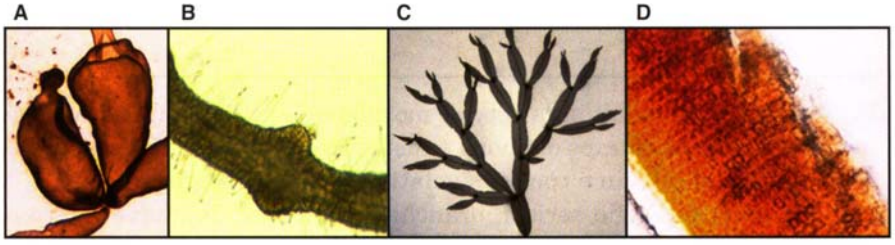

Figure 13-4 *Boldia erythrosiphon*, a hollow saccate tissuelike thallus (A); *Lemanea fluviatilis*, a cartilaginous tubular thallus (B); *Caloglossa leprieurii*, a dichotomously divided blade with a central midrib (C); and *Hildenbrandia angolensis*, a crustose red alga with short files of cells (D)

outer assimilatory filaments (Figure 13-3). Other morphological forms include tissue-like saccate thalli, flat blades, and cartilaginous tubes as well as crusts, which consist of appressed, short, vertical files of cells (Figure 13-4).

The freshwater red algae exhibit asexual reproduction, ranging from cell division in the unicells to archeospore production in many forms. Sexual reproduction is common in many of the morphologically complex forms and is usually part of an alternation of generations. In most species, the larger and more recognizable form is the haploid gametophyte, which produces the gametangia at maturity. The widespread genus *Batrachospermum* is a good example of this phenomenon with carpogonia (female gametangium) and spermatangia (male gametangium) often on the same plant

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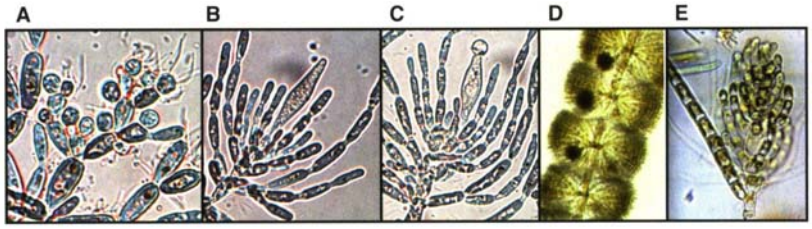

Figure 13-5 *Batrachospermum gelatinosum* reproduction showing colorless ovoid spermatangia at branch tips (A); carpogonium with a lance-shaped trichogyne (B), fertilized carpogonium (C); gametophyte whorls containing carposporophytes appearing as dense spherical masses (D); and a chantransia stage filament with an attached young gametophyte with whorled branching (E)

(monoecious), though occasionally on separate plants (dioecious) (Figure 13-5). The spermatia (male gametes) are released into the flowing water and attach to the tip of the carpogonium (the trichogyne) at which time fertilization takes place. The fertilized carpogonium divides to form a small diploid stage called the caroposporophyte, which appears as dense spherical masses among the vegetative branches (Figure 13-5). This stage releases diploid carpospores that germinate into a typically microscopic, simply branched stage, the chantransia, which forms a new gametophyte directly attached via meiosis (Figure 13-5).

A recent monograph by Kumano (2002) has summarized much of the worldwide literature on the taxonomy of freshwater Rhodophyta. There is an older monograph on North American freshwater red algae by Flint (1970), but this book is difficult to obtain and is out of date in terms of the systematics. The taxonomy of these algae is based on the vegetative and reproductive features noted above and some additional ones and is somewhat in a state of flux, based on newer information derived from DNA sequence analyses. For example, a new genus and species, *Petrohua bernabei*, has been recently described from Chile (Vis et al. 2007). In North America, there are currently recognized ~65 infrageneric taxa and 22 genera (Sheath 2003), though these figures are likely to change somewhat. The genus *Batrachospermum* is the most diverse, with 26 infrageneric taxa, but this genus is considered to be unresolved in terms of relationships among groupings in various gene trees (e.g., Vis et al. 2007). Recently, Entwisle et al. (2009) have started the process of dividing *Batrachospermum* by creating a new genus, *Kumanoa*, from one of the former sections.

KEY TO GENERA

The following is a key to the orders of freshwater red algae of North America as they are currently conceived and based on easily recognizable features, including recent changes proposed by Pueschel et al. (2000), Saunders and Necchi (2002), and Yoon et al. (2006):

1. Thallus unicellular, Porphyridiales

1. Thallus multicellular, 2

2. Thallus pseudofilamentous, Stylonematales

2. Thallus filamentous, tissuelike, or crustose, 3

3. Thallus crustose, Hildenbrandiales

3. Thallus filamentous or tissuelike, 4

4. Mature filaments uniseriate at the base and multiseriate at the apex, Bangiales

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4. Mature thallus without a change in number of cell series, 5
5. Sexual reproduction by archeospores formed by an unequal, oblique division, Compsopogonales
5. Asexual reproduction by archesopores formed by differentiation in apical sporangia or nonexistent, 6
6. Spermatangia borne on elongate, differentiated cells, Balbianiales
6. Spermatangia borne on undifferentiated vegetative cells and/or carpogonial branches, 7
7. Multiseriate main axis, Thoreales
7. Uniseriate main axis, 8
8. Thalli in short, dense tufts, Acrochaetiales
8. Thallus semierect filamentous or tissuelike, 9
9. Gametophyte formed attached to a microscopic chantransia stage, Batrachospermales
9. Gametophyte free living, Ceramiales

The freshwater North American genera of each order are as follows:

- Acrochaetiales: *Audouinella*
- Balbianiales: *Rhododraparnaldia*
- Bangiales: *Bangia* (also known as *Bangiadulcis*, see Nelson 2007)
- Batrachospermales: *Balliopsis* (formerly known as *Ballia*, see Saunders and Necchi 2002), *Batrachospermum* (in part also known as *Kumanoa* for section *Contorta*; see Entwisle et al. 2009), *Lemanea*, *Paralemanea*, *Sirodotia*, *Tuomeya*
- Ceramiales: *Bostrychia*, *Caloglossa*, *Polysiphonia*
- Compsopogonales: *Boldia*, *Compsopogon*
- Hildenbrandiales: *Hildenbrandia*
- Porphyridiales: *Flintiella*, *Porphyridium*
- Stylonematales: *Chroodactylon*, *Chroothece*, *Kyliniella*
- Thoreales: *Nemalionopsis*, *Thorea*

The common genera are differentiated later in the Identification section.

ECOLOGY

Red algae are primarily marine, with only approximately 3% of the more than 5,000 species occurring in freshwater habitats that would constitute source water (Sheath 1984). Most of the inland rhodophytes are restricted to streams and rivers, though some species can be collected in lakes, on moist soil, in sloth hair, and on cave walls.

In North America, 51% of 1,000 first- to fourth-order stream reaches surveyed contain red algae, and 24% have two or more species (Sheath and Hambrook 1990, Sheath 2003). The maximum number of species collected per reach is six. The most widespread species is *Batrachospermum gelatinosum*, which occurs from polar desert on Ellesmere Island in Nunavut to the southeastern coastal plain in Louisiana.

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Members of the Compsopogonaceae, Thoreales, Hildenbrandiales, Ceramiales, and *Kumanoa* (*Batrachospermum* section *Contorta*) are largely restricted to south temperate to tropical streams. *Boldia* and *Tuomeya* have been collected only in eastern North America, ranging from Ontario and Quebec to the southeastern United States.

Most riverine red algae occur in moderate flow regimes (mean 29–57 cm sec^{-1}) (Sheath and Hambrook 1990). Moderate flow enhances primary productivity, pigment levels, respiration rates, and nutrient uptake rates as well as causing washout of loosely attached competitors. Flowing waters also replenish nutrients and gases and reduce boundary layers and the zone of depletion around the thallus. Some freshwater red algae, such as *Lemanea* and *Paralemanea,* are common in stream reaches with high current velocities (>1 m sec^{-1}). Sheath and Hambrook (1990) calculated mean potential velocities (in cm sec^{-1}) at which different morphological forms of red algae would break: tufts 80; mucilaginous filaments, 160; and cartilaginous tubes, 580.

The light regime, which includes changes in intensity, quality, and photoperiod, is one of the major factors affecting the distribution and seasonality of freshwater red algae (Sheath and Hambrook 1990). Illumination affects algal growth by photosynthesis, processes indirectly related to photosynthesis, and those processes unrelated to photosynthesis. In deciduous forested regions, the surrounding tree canopy can reduce light penetration in a shaded reach by 90%–99%, and freshwater rhodophytes, such as *Batrachospermum boryanum*, disappear during periods of peak shading. Localized distribution in a drainage basin will also be affected by the light regime, and red algae tend to be most abundant in mid-order reaches.

Temperature influences the latitude, elevation, and drainage basin distribution patterns of freshwater red algae (Sheath and Hambrook 1990). In North America, the greatest species diversity has been found in temperate and tropical latitudes. Certain taxa are most commonly distributed in tropical and subtropical regions, as noted above. In contrast, the genus *Lemanea* is widespread in boreal and alpine habitats. In temperate regions, most red algae are restricted to elevations less than 900 m above sea level, and they exhibit their maximum biomass, growth, and reproduction from late fall to early spring. This seasonality is related to temperature but probably more so to light and nutrient availability.

In general, red algae occur in stream reaches that have low to moderate nutrient levels and are good indicators of relatively little disturbed sites. Flow replenishment and reduction of the boundary layer are factors accounting for this distribution, and many freshwater red algae can produce colorless hair cells that can increase the surface area for nutrient uptake (Figure 13-4) (Sheath and Hambrook 1990). Most inland red algae are also localized in reasonably unpolluted waters and are infrequent to absent in streams and rivers that are organically enriched, silted, or high in metal ions. Exceptions to metal intolerance are *Lemanea fluviatilis* and *Bangia atropurpurea.*

The interaction between pH and the form of inorganic carbon greatly influences the distribution of freshwater red algae (Sheath and Hambrook 1990). Many species most frequently occur between pH 6 and 7, a range in which free carbon dioxide is reasonably plentiful. The majority of these species is restricted to freshwater habitats and can be classified as specialists in terms of habitat (~50 species in North America) (Sheath 2003). Another group of inland rhodophytes appear to invaders from marine and estuarine habitats and typically occur at a higher pH range and moderate to high conductance values (~9 species in North America).

Thirty-eight animals have been observed to ingest freshwater red algae, based on gut content or feeding studies (Sheath and Hambrook 1990, Sheath 2003). These grazers include amphipods, caddis fly and stonefly larvae, chironomids, snails, and cyprinoid fish. Cases of larvae and pupae from six caddis fly species have been observed

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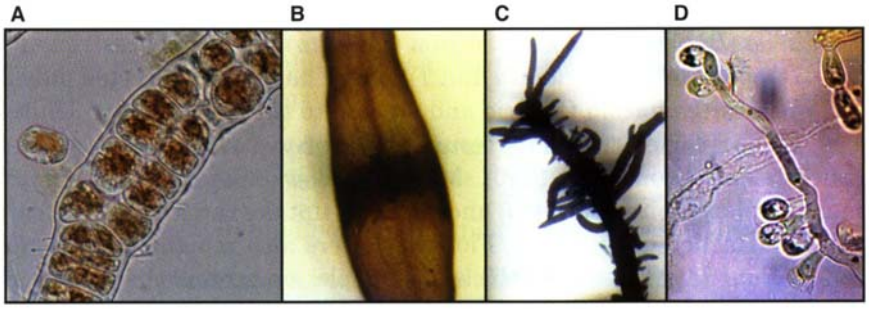

Figure 13-6 *Bangia atropurpurea*, a filament with a unsiseriate base and multiseriate apex (A); *Paralemanea catenata*, a cartilaginous tubular thallus with a prominent ring of spermatangia (B); *Tuomeya americana*, a cartilaginous filament with compacted whorls (C); and *Sirodotia suecica* carposporophyte that creeps along the main axis (D)

to contain pieces of freshwater rhodophyte thalli in North America. Three genera of chironomids have also been observed to incorporate pieces of red algae in their cases.

Competition for suitable substrata can occur among species of freshwater red algae or with other benthic algae in streams and rivers (Sheath and Hambrook 1990, Sheath 2003). Low-growing forms, such as the chantransia stage of the Batrachospermales and Balbianiales, *Audouinella* and *Hildenbrandia*, are components of the stream epilithic community and compete with a complex periphyton association usually dominated by diatoms. The semierect forms compete with macrophytes, such as bryophytes, as well as other taxa of macroalgae.

SIGNIFICANCE

In terms of significance to water treatment, freshwater red algae are not problematic because they are largely indicators of good to reasonable water quality and do not form toxins or odors in water supplies.

IDENTIFICATION

The widespread members of the orders presented above will be differentiated in the following keys.

Bangiales

Only one genus, but it is widespread in the lower Laurentian Great Lakes (Müller et al. 2003).

1. Thallus filamentous at maturity with uniseriate base and multiseriate apex, *Bangia* (Figure 13-6)

Batrachospermales

1. Thallus in the form of tissuelike cartilaginous tubes, 2

1. Thallus filamentous, 3

2. Spermatangia in distinct patches, central axis uncorticated, *Lemanea* (Figure 13-4)

2. Spermatangia in rings, central axis corticated, *Paralemanea* (Figure 13-6)

3. Filament cartilaginous with densely packed lateral whorls, *Tuomeya* (Figure 13-6)

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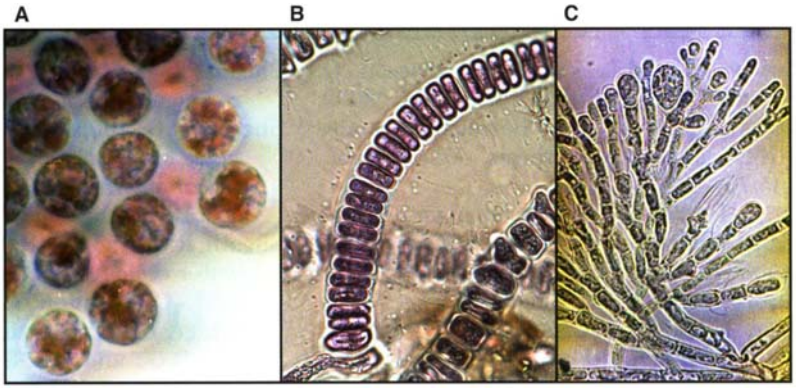

Figure 13-7 *Flintiella sanguinaria*, unicell with a lobed, parietal chloroplast (A); *Kyliniella lativica*, a reddish pseudofilament with parietal chloroplasts (B); and *Nemalionopsis tortuosa*, multiseriate filament with peripheral archeosporangia (C)

3. Filament not typically cartilaginous and whorls distinguishable in at least younger portions of the thallus, 4

4. Carposporophyte distinct compact mass, *Batrachospermum* (including *Kumanoa*) (Figure 13-5)

4. Carposporophyte branched filament creeping along the main axis, *Sirodotia* (Figure 13-6)

Ceramiales

None of the genera listed are very widespread.

Compsopogonales

1. Thallus a monstromatic sac, *Boldia* (Figure 13-4)

1. Thallus a corticated, branched filament, *Compsopogon* (Figure 13-3)

Porphyridiales

1. Unicell with an axial, stellate chloroplast with a prominent pyrenoid, *Porphyridium* (Figure 13-1)

2. Unicell with lobed, parietal chloroplast without a pyrenoid, *Flintiella* (Figure 13-7)

Stylonematales

1. Cells enclosed in a layered gelatinous matrix, *Chroothece*

1. Gelatinous matrix not layered, 2

2. Bluish cells with an axial stellate chloroplast, *Chroodactylon* (Figure 13-1)

2. Reddish cells with parietal chloroplasts, *Kyliniella* (Figure 13-7)

Thoreales

1. Archesporangia central along axis, *Thorea* (Figure 13-3)

2. Archeosporangia peripheral in the thallus, *Nemalionopsis* (Figure 13-7)

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REFERENCES

Entwisle, T.J., M.L. Vis, W.B. Chiasson, O. Necchi Jr., and A.R. Sherwood. 2009. Systematics of the Batrachopsermales (Rhodophyta): A synthesis. *Jour. Phycol.*, 45:704–715.

Flint, L.H. 1970. *Freshwater red algae of North America.* New York: Vantage Press.

Kumano, S. 2002. *Freshwater red algae of the world.* Bristol, UK: Biopress.

Müller, K.M., K.M. Cole, and R.G. Sheath. 2003. Systematics of *Bangia* (Bangiales, Rhodophyta) in North America. II. Biogeographical trends in karyology: Chromosome numbers and linkage with gene sequence trees. *Phycologia*, 42:209–219.

Nelson, W.A. 2007. *Bangiadulcis* gen. nov.: A new genus for freshwater filamentous Bangiales (Rhodophyta). *Taxon,* 56:883–886.

Pueschel, C.M., G.W. Saunders, and J.A. West. 2000. Affinities of the freshwater red alga *Audouinella macrospora* (Florideophyceae, Rhodophyta) and related forms based on SSU rRNA gene sequence analysis and pit plug ultrastructure. *Jour. Phycol.*, 36:433–439.

Saunders, G.W., and O. Necchi Jr. 2002. Nuclear rDNA sequences from *Ballia prieurii* support recognition of *Balliopsis* gen. nov. in the Batrachospermales (Florideophyceae, Rhodophyta). *Phycologia*, 41:61–67.

Sheath, R.G. 1984. The biology of freshwater red algae. *Phycolog. Res.,* 3:89–157.

Sheath, R.G. 2003. Red algae. In *Freshwater algae of North America: Ecology and classification.* J.D. Wehr and R.G. Sheath, eds. San Diego: Academic Press.

Sheath, R.G., and J.A. Hambrook. 1990. Freshwater ecology. In *Biology of the red algae.* K.M. Cole and R.G. Sheath, eds. Cambridge, UK: Cambridge University Press.

Sheath, R.G., and A.R. Sherwood. 2002. Phylum Rhodophyta. In *The freshwater algal flora of the British Isles.* D.M. John, B.A. Whitton, and A.J. Brook, eds. Cambridge, UK: Cambridge University Press.

Vis, M.L., J.T. Harper, and G.W. Saunders. 2007. Large subunit rDNA and *rbc*L gene sequence data place *Petrohua bernabei* gen. et sp. nov. in the Batrachospermales (Rhodophyta), but do not provide further resolution among taxa of this order. *Phycolog. Res.*, 55:103–112.

Yoon, H.S., K.M. Müller, R.G. Sheath, F.D. Ott, and D. Battacharya. 2006. Defining major lineages of red algae (Rhodophyta). *Jour. Phycol.*, 42:482–492.

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Section III

Management

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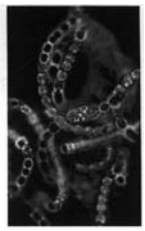

Chapter 14

Source Water Assessment and Control/Treatment Strategies for Harmful and Noxious Algae

JoAnn Burkholder, William Frazier, and Meghan B. Rothenberger

SUMMARY

This chapter considers source water assessment and protection from the perspective of noxious algal growth (here, including cyanobacteria or blue-green algae), and treatment strategies for removing algal cells, toxins, and other undesirable bioactive substances. Examples are also presented of advanced technologies and approaches that are being applied to improve assessment, treatment (at potable water treatment plants), control (in surface source waters), and early warning systems for safeguarding water supplies from noxious algae. Many species of cyanobacteria, chlorophytes, and ochrophytes in surface waters can impair water supplies through toxin production, taste and odor problems, and/or clogging of open-air distribution systems. Most have been shown to be strongly stimulated by nutrient (phosphorus and nitrogen) pollution, underscoring the importance of proactive source water protection as the most effective approach to minimize noxious and harmful algae, and the importance of N and P co-management.

Real-time remote monitoring technology has strengthened source water assessment of pollutant inputs and toxic cyanobacterial blooms. Pollutant source tracking has also been advanced by techniques such as stable isotope analyses and by molecular detection assays for organisms such as fecal coliphages that indicate human versus

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animal wastes. Other molecular techniques have made it possible to discern actively toxic versus nontoxic strains of cyanobacteria in natural bloom samples. Various models have been developed to assess the effects of changing watershed land use practices on pollutants from airsheds and on surface and groundwater quality. The water quality models typically use a mass balance approach, which attempts to account for all changes in the mass of a parameter caused by physical, chemical, and biological processes to predict how a system will respond to land use changes or improved management practices. It has proven difficult, however, to measure many of the relevant hydrologic, geochemical, and biological parameters with any certainty. More recently, decadal changes in land use and influences on water quality have been examined with longitudinal data analysis as an important first step in the integration of Geographic Information Systems (GIS) analysis and watershed models. This empirical approach also provides a powerful tool to address watershed management issues and explore/evaluate management alternatives. In addition, powerful ordination techniques such as nonmetric multidimensional scaling are beginning to be applied to assess potential environmental predictors of changes in phytoplankton groups (e.g., cyanobacteria) or harmful species of interest under chronic eutrophication.

Once a water source becomes prone to undesirable algal blooms, various mitigative measures can be applied. Physical, chemical (herbicide), and biological techniques used in attempts to control harmful algae are limited, however, and have not substantially improved over the past several decades. The degree of success depends on factors such as the size and natural or imposed flushing and mixing rates in the affected area, the depth and volume of the waterway, the algal species involved, internal nutrient loading characteristics, and the nutrient loading reductions attained. Treatment of drinking water supplies is increasingly a challenge, since aggressive watershed development by urbanization, industrialized agriculture, and other sources of pollution is forcing many communities to rely upon increasingly degraded sources. As general guidance for drinking water treatment, intracellular cyanotoxins can be removed effectively by conventional treatment (coagulation and sedimentation followed by filtration) and membrane filtration, especially reverse osmosis, nanofilters, and ultrafilters. Several oxidants effectively inactivate some cyanotoxins depending upon the pH, temperature, and toxin concentration. Treatment to remove taste and odor compounds often includes oxidation (ozone, chlorine, chlorine dioxide) with activated carbon adsorption. Beyond attempts to protect source waters, a multibarrier, multistep mitigative approach should also encompass distribution systems and education of water utility personnel, policy makers, and the general citizenry about issues involving water quality impairment from noxious and harmful algae.

While significant progress has been made in the past decade to assess and combat nutrient pollution, algal blooms, and their toxins and taste and odor compounds, many frontiers remain. High-quality long-term data sets and improved land use/water quality models and ordination analyses are needed to more accurately estimate nutrient loadings to source waters and to accurately predict the effects on harmful algae. Environmental, physiological, and molecular controls on the production of taste and odor substances and toxins need to be better understood. Basic research is needed to develop new and/or improved methods for eliminating anatoxin-a, saxitoxins, cylindrospermopsin, and their derivatives in water treatment processes. Other important research should also be strongly supported on the development/refinement of molecular approaches and other technologies that will enable rapid, reliable screening for cyanotoxin detection and quantification. Finally, centralized national and international

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databases are needed for information on noxious and harmful freshwater algal blooms, to improve understanding about the factors that control bloom initiation and development, and to help the general citizenry and policy makers understand the economic impacts of noxious and harmful algal blooms, the formidable challenges they represent for water utility and natural resource managers, and the need to provide the resources to water utilities to meet these expanding challenges.

INTRODUCTION

Many species of cyanobacteria, chlorophytes, and ochrophytes in surface waters can impair water supplies through toxin production, taste and odor problems, and/or clogging of open-air distribution systems. They mainly include planktonic and benthic cyanobacteria; certain filamentous, coenobic, and tissue-forming chlorophytes; an array of problematic taste and odor taxa including planktonic and benthic cyanobacteria, chlorophytes, and ochrophytes; and the benthic mat-forming diatom *Didymosphenia geminata* in streams and rivers. Their detrimental ecological and socioeconomic effects can include production of potent toxins and other bioactive substances; habitat degradation leading to fish kills and related water quality impairment; increased fouling of pumps, filters, and intake pipes; compromised potable water supplies; increased costs of water treatment; and other economic loss.

Most noxious and toxic freshwater algae (here, including cyanobacteria or blue-green algae) have been shown to be strongly stimulated by nutrient overenrichment (Burkholder 2009). They can also be influenced by co-occurring pollutants such as suspended sediments and various chemical environmental contaminants. While surface waters are generally more vulnerable to contamination than groundwater, underground burial of pollutant sources and percolation of pollutants such as nitrate through soil into the water table has resulted in contamination of groundwater in many areas throughout the United States. Groundwater springs contribute to lake and reservoir nutrient inputs, and groundwater can also seasonally represent a major proportion of the flow in mid- and lower river segments where noxious algal blooms can commonly occur. Thus, both surface waters and groundwater can be important sources of nutrients and other pollutants affecting algal growth.

As burgeoning human population growth and watershed development increasingly stress the quantity and quality of available water supplies, source water protection is an ever more formidable challenge. Source water quality is highly variable and can quickly change with storm events (minutes to hours) and watershed changes. Assessment of the many known toxins and other bioactive substances such as taste and odor compounds produced by cyanobacteria and various other algae, and prioritization for management actions to minimize a wide range of impacts can be a daunting task, with few universally applicable tools. On the other hand, from a positive perspective, various technologies under ongoing development are beginning to allow rapid, routine detection of multiple potentially toxic cyanobacteria and some of their toxins. In addition, some new technologies affect algal toxin inactivation and removal. This chapter considers basic tenets of source water assessment from the perspective of controlling noxious algal growth, and treatment strategies for removing the cells, toxins, and other undesirable bioactive substances. Examples are also presented of advanced technologies and approaches that are being applied to improve source water assessment and early warning systems for safeguarding water supplies from noxious algae.

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SOURCE WATER ASSESSMENT

Watershed Analysis: Land Use and Airshed Influences on Water Quality

Human activities such as urban development and intensification of agriculture, driven by the need to provide resources for the expanding human population, are profoundly and pervasively changing the world landscape (Rothenberger 2007). Land use change in drainage basins often results in decreased vegetation and conversion of valuable wetlands to croplands or housing developments with more impervious surfaces. Terrestrial vegetation and wetlands act as filters that moderate flow and retain or assimilate nutrients during their downstream passage to the sea (Mitsch and Gosselink 2000). Their replacement with urban and agricultural land use practices has increased runoff and export of nutrients and other pollutants. As a result, many surface waters have been transformed from balanced, productive ecosystems to systems that are sustaining nutrient overenrichment, biogeochemical alterations, biological community disturbances, food web instability, and stimulation of undesirable species as the ecosystems become more imbalanced (National Research Council 2000).

Nutrient inputs that can encourage the growth of harmful and noxious algae in freshwaters include both inorganic (ammonium, nitrate, phosphate) and organic (dissolved organic and particulate matter) forms of nitrogen and phosphorus. Certain forms of dissolved organic carbon can also stimulate some harmful species. In rivers or run-of-river impoundments with appreciable suspended sediments, however, light may be the primary resource limiting algal growth, with nutrients of secondary importance. In such systems, algae are able to take advantage of nutrient additions if there is a long enough duration between episodic sediment loading events caused by storms or land development (Burkholder 1992). In addition to the form and concentration of nutrient (N, P) species, the supply ratios and the timing and intensity of nutrient loading strongly influence the extent to which noxious algal blooms will be stimulated (Hecky and Kilham 1988, Wetzel 2001).

Numerous human activities can directly or indirectly add nutrients to water supplies through surface waters, groundwater, and atmospheric deposition. These include urban/suburban runoff, agricultural runoff and atmospheric loading, sewage discharges, septic tank leachate or overflows, and various industrial/manufacturing discharges (Tables 14-1 and 14-2). The US Environmental Protection Agency (USEPA) requires states to delineate or map source water protection areas (land areas that could contribute water and pollutants to the water supply) for sources of public drinking water, including watershed contamination source inventories and susceptibility analyses (USEPA 1997). USEPA considers three major steps in source water assessment: (1) delineation of the source water assessment area; (2) completion of an inventory of potential sources of contamination (here, focusing mainly on nutrient pollution); and (3) determination of the susceptibility of the water supply to contamination (see http://cfpub.epa.gov/safewater/sourcewater/sourcewater.cfm?action=Assessments).

Although most algal species are cosmopolitan, human activities have contributed to the distribution and proliferation of many harmful taxa. An interesting example is provided by the benthic stream-inhabiting diatom, *D. geminata*. Historically it was native to nutrient-poor lakes and streams of northern latitudes, but recently it has acted as an invasive species in streams and rivers within its native range and has also expanded its range to the Southern Hemisphere (Spaulding and Elwell 2007).

Algal taste and odor compounds have sometimes been linked to source water degradation, but the causes and controls of their production are poorly understood. Some algal taste and odor compounds are synthesized during normal growth, but others

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Table 14-1 Considerations for watershed contamination source inventories, emphasizing nutrient pollution and susceptibility analyses of groundwater and surface water sources of drinking water (Source: Adapted from USEPA 1997)

- Up-to-date land-use inventory (point and nonpoint sources of suspended sediments, nutrients, and other chemical environmental contaminants, or CECs*)
- Existing best management practices (BMPs) or controls
- Hydrology: surface/groundwater interactions
- On-site landfilling, land treating, surface impounding of wastes, and illicit dump sites
- Sand/gravel excavations that expose the water table
- Sludge disposal areas
- Permitted (NPDES†) major and minor point sources
- Industrial wastewater discharges into on-site drainage systems (e.g., septic systems, dry wells)
- Other septic systems, cesspools
- Agricultural, landscaping, or golf course activities (nutrient and other CEC release to groundwater or stormwater runoff to surface waters)
- Areas with impervious cover (urban, suburban) that can contribute nutrients (e.g., from pet wastes) and/or increase stormwater runoff
- Natural sources (e.g., wildlife)
- Other stormwater discharge locations
- Construction activities
- Depth to the water table
- Nature of the aquifer (confined vs. unconfined)
- Hydrogeologic/soil/chemical/physical factors that influence the likelihood of ground- and surface water contamination, and the transport/fate of nutrients from fertilizers and other sources
- Well integrity (considering high nitrate)
- Presence of recreational areas or greenway trails near a drinking water source
- Waters that are known to be impaired by nutrient pollution (basis: 303d list, water quality monitoring, and use assessments in states' 305[b] reports)

* Chemical environmental contaminants—fertilizers, pesticides, heavy metals, pharmaceuticals, etc.
† NPDES—National Pollutant Discharge Elimination System.

Table 14-2 Some direct and indirect sources of nutrient overenrichment in watersheds (Source: Adapted from USEPA 1997). USEPA's 305(b) guidance requests states to identify major sources of contamination for waters designated as potable supplies.

- Agriculture—crop production, pasture and range grazing, concentrated or confined animal feed operations
- Atmospheric deposition
- Collection system failure
- Combined sewer overflow
- Golf courses
- Groundwater loadings, withdrawals
- Hydromodification—channelization, dredging, dams, upstream impoundment, flow regulations/modifications
- Other habitat modifications—removal of riparian vegetation, bank or shoreline modification/ destabilization, drainage or filling of wetlands
- Industrial point sources
- Internal nutrient cycling, sediment resuspension (larger rivers, lakes, reservoirs)
- Land disposal (sludge, wastewater, landfills, debris)
- Land disturbance (erosion, construction, mining)
- On-site wastewater systems (septic tanks), septage disposal
- Hazardous wastes
- Leaking above- and belowground storage tanks, sewer lines
- Marinas and recreational boats (in-water and on-land releases)
- Municipal point sources (major and minor sewage treatment plants, package plants*)
- Natural sources (e.g., geological phosphate deposits, shorebirds, wildlife)
- Silvaculture (harvesting, restoration, residue management, and related point sources)
- Forest management (e.g., pumped drainage, fertilizer and pesticide applications)
- Urban/road/bridge runoff/storm sewers
- Waste storage areas (leaks)

* Smaller, private wastewater treatment plants.

are produced when cellular integrity is compromised (Wnorowski 1992, Jüttner 1995, Watson 2004). Peroxidation reactions induced by light in combination with heavy metals or pesticides can also be involved; thus, common chemical control of cyanobacteria blooms with copper sulfate or other herbicides can sometimes promote production of strong odorous compounds. Watson (2004) wrote, "These chemicals can be symptomatic

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of short-term problems with source, treatment or distribution systems. At a broader level, they can signify fundamental changes in aquatic ecosystems induced by human activity...linked with the widespread eutrophication of many surface waters; however, there has been a recent growth in the number of T/O [taste and odor] events reported in oligomesotrophic [nutrient-poor or only moderately nutrient-enriched] systems...." In general, nonetheless, algal polyunsaturated fatty acid (PUFA) derivatives have occurred most frequently and in highest abundance in eutrophic systems, suggesting that changes in odorous compounds may have potential in assessing source water and basin integrity (Watson 2004).

Freshwater cyanobacteria blooms are strongly associated with nutrient over-enriched (eutrophic), poorly flushed waters and phosphorus loading (Schindler 1977, Hecky and Kilham 1988). While phosphorus is considered the more important nutrient limiting algal growth in freshwaters, nitrogen can also be stimulatory (Figures 14-1 through 14-3). Cyanobacteria blooms create aesthetic problems, often deplete the dissolved oxygen in the water at night, and cause fish kills (Whitton and Potts 2000). Many species of cyanobacteria are also capable of producing toxins (toxic strains or populations; see High-throughput Analysis of Cyanotoxins section) and other undesirable bioactive substances such as taste and odor compounds (Chorus and Bartram 1999). The effects of P on growth and toxicity of cyanobacteria are complex and vary among species and among strains within a given species (Zurawell et al. 2005). Nevertheless, reduction of bioavailable phosphorus and nitrogen (P and N_i co-management), while often economically and politically difficult to achieve, remains the most effective long-term approach for protecting source waters.

Real-time Remote Monitoring Systems

Established real-time remote monitoring (RTRM) technology provides the ability to detect and report the earliest stages of changes imposed by events such as pollutant inputs, toxic cyanobacterial blooms, sewage leaks, hazardous runoff, fish kills, and bioterrorism activity (Glasgow et al. 2004). It strengthens early warning capability by enabling water quality managers to identify serious problems quickly and avoid even more serious risks to the economy and public health (Reed et al. 2010).

Automated platform stations have been designed and improved over the past decade to detect changes in environmental conditions (temperature, pH, dissolved oxygen, conductivity), turbidity, and algal blooms as chlorophyll *a* and phycocyanin. The latter pigment can be used (absorbance 630 nm), together with a cursory check of water samples at low power under light microscopy, to indicate developing or established cyanobacterial blooms (Reed et al. 2010). Certain carotenoid photopigments have also been used as biomarkers for cyanobacteria, especially myxoxanthin (for coccoid forms) and aphanizanthin (for filamentous forms) (Ritchie and Zimba 2005, de la Cruz et al. 2007). The automated systems provide data in real or near-real time, 24/7, and can be set to an hourly frequency or longer as desired. Depth profiling capability can be especially valuable to managers of water utilities that can vary their intake depth to, for example, minimize treatment of low-oxygen water or contamination by algal blooms and turbidity (e.g., Figure 14-4).

RTRM systems can be positioned at areas of potential "hot spots" for contamination of a potable water supply, for example, at tributaries draining sub-watersheds known to carry high pollutant loads. They can be programmed to trigger an alarm for events related to a sudden drop in pH that may be associated with a precipitation/runoff event or a sudden increase in turbidity. They can be equipped with automated

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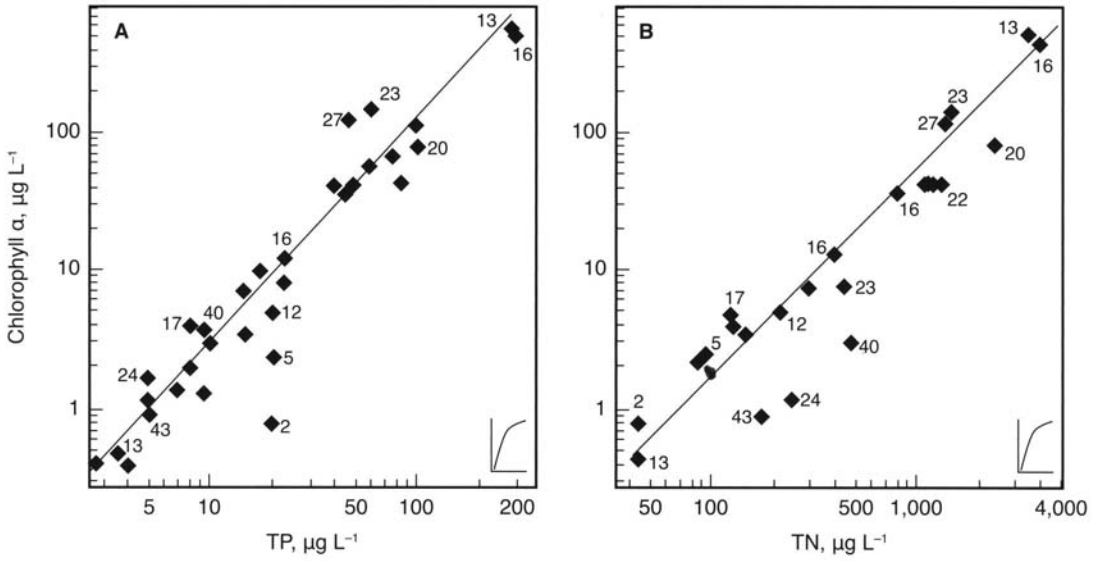
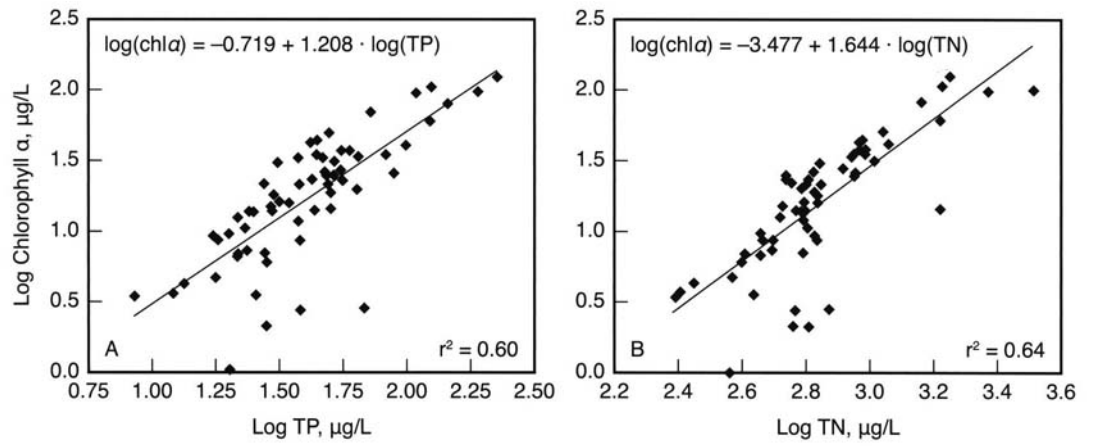


NOTE: Numbers next to points represent the N:P ratio (by mass). Note that lakes with exceptionally low and exceptionally high N:P ratios are outliers in the TP-chl*a* and TN-chl*a* relationships, respectively.

Figure 14-1 Relationship between chlorophyll *a* (chl*a*) content and (a) total phosphorus, TP, or (b) total nitrogen, TN, in surface and near-surface waters of natural lakes in Japan during summer. (Modified by Sakamoto [1966] in Kalff [2002, *Limnology*, Prentice Hall], with permission from Pearson Education Inc.)



NOTE: The positive relationship between chl*a* and both TN and TP was supported by correlation analyses and hierarchical ANOVA models. The models also indicated significant positive relationships between TN and TP, and between SS and both TP and TN. Agricultural land use was positively correlated with TKN (total Kjeldahl nitrogen) for the reservoirs considered collectively, and with TN, TKN, TP, and chl*a* in model reservoirs. Cyanobacteria comprised 60%–95% (up to 1.9 × 106 cells mL^{-1}) of the total phytoplankton cell number. Potentially toxic taxa were dominated by *Cylindrospermopsis raciborskii* and *C. philippinensis*. Although known microcystin producers were low in abundance, microcystin (<0.8 µg L^{-1}) was detected in most samples. TP and chla were significant predictors of total cyanobacterial abundance.

Figure 14-2 Linear regression analysis showing the relationship between log(chlorophyll *a*, chl*a*) and (A) log(total phosphorus, TP) and (B) log(total nitrogen, TN) for 11 meso-eutrophic, turbid run-of-river impoundments (means 25–125 µg TP L^{-1}, 410–1,800 µg TN L^{-1}, 3–70 µg chl*a* L^{-1} and 5.7–41.9 mg suspended solids [SS] L^{-1}). (Source: Touchette et al. [2007, *Lake and Reservoir Management*], with permission from the North American Lake Management Society.)

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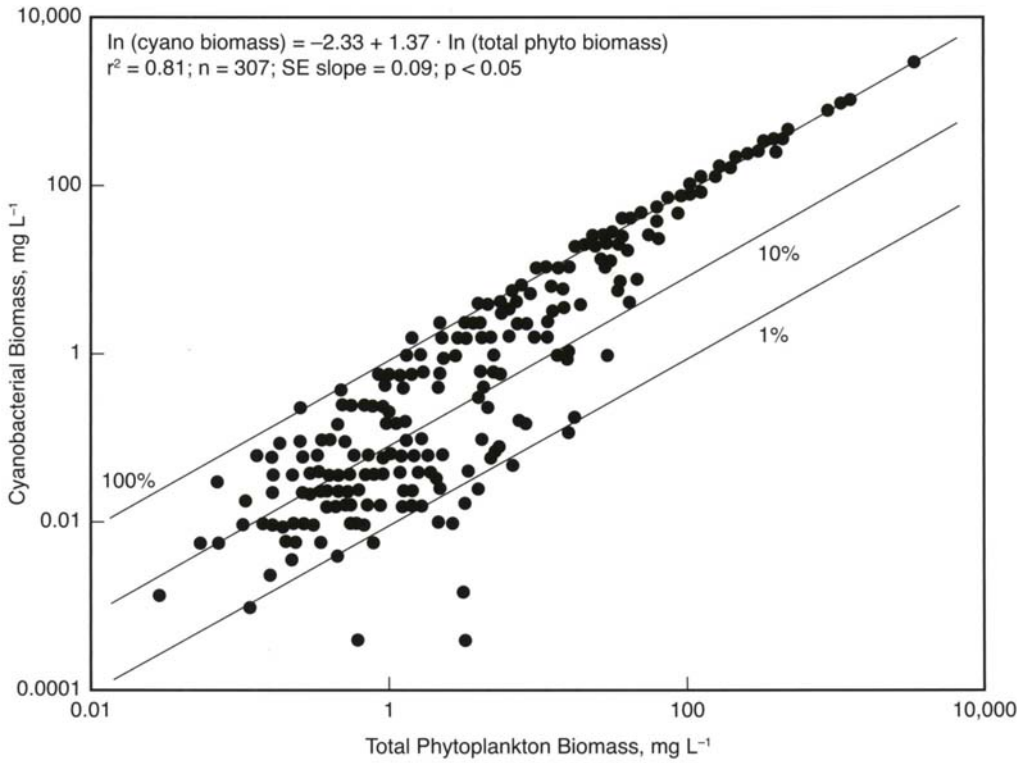
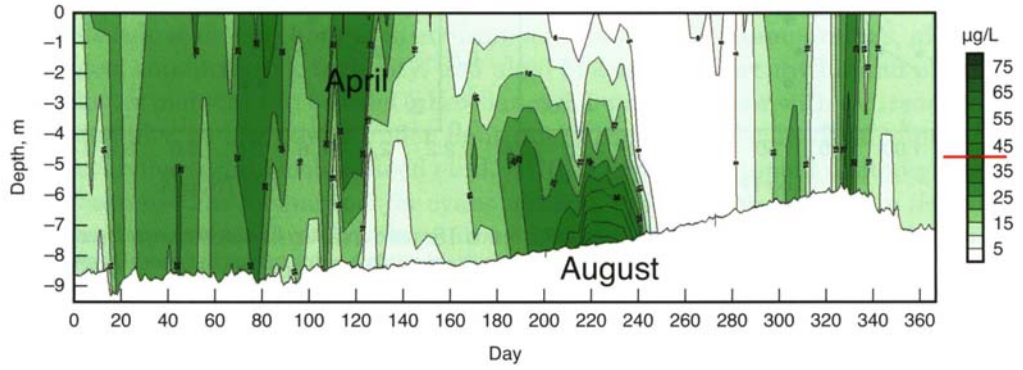


NOTE: The solid lines indicate the proportion of phytoplankton biomass comprised by cyanobacteria as 100%, 10%, and 1%. Note that cyanobacteria represent an increasing proportion of the total phytoplankton biomass as total biomass increases. In addition, cyanobacteria can contribute variable portions of the total phytoplankton biomass when the total is less than ~50 mg L^{-1}, but they represent ~100% of the total in highly eutrophic lakes (total phytoplankton biomass more than 100 mg L^{-1}).

Figure 14-3 Relationship between cyanobacteria biomass (fresh weight) and total phytoplankton biomass (fresh weight) in 165 Florida (US) lakes (Source: Adapted from Canfield et al. [1989] in Kalff [2002, *Limnology*, Prentice Hall], with permission from Pearson Education Inc.)



Note: In this warm temperate climate, phytoplankton blooms commonly occur in spring–early summer. Cyanobacteria, including potentially toxic species, contributed ~75%–90% of the phytoplankton cells during blooms. These data assisted water treatment plant operators in selecting the optimal depth for the intake pipe to minimize contamination by cyanobacteria and their toxins.

Figure 14-4 Example of continuous data from RTRM: Depth versus time contours of chlorophyll (as relative fluorescence units) during January to mid-November 2006 in a major potable water source of North Carolina, the Falls Lake impoundment, near the intake of the City of Raleigh's potable water treatment plant

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Isco® water samplers for periods of potentially high chemical pollutant or pathogenic microbe loading. RTRM systems can also be augmented with acoustic Doppler current profilers to track incoming volume of flow and improve assessment of pollutant loadings. These systems are adaptable for additional sensors as they become available.

Stable Isotopes to Track Nitrogen Pollution

Stable (nonradioactive) nitrogen, carbon, and oxygen isotopes can be valuable in providing a diagnostic "signature" to verify contamination by one to a few major pollutant sources that occur in close proximity to a water body. Isotope abundance is measured using a mass spectrometer. The stable isotope composition of a given sample is expressed as a ratio relative to an internationally accepted reference standard:

$$\delta^{xx}E = 1{,}000 \cdot \left[\frac{R_{sample}}{R_{standard}} - 1 \right], ‰$$

where E is the element of interest (^{15}N, ^{13}C, ^{18}O), ^{xx} is the mass of the rarer (and heavier) isotope in the abundance ratio, and R is the abundance ratio of those isotopes (e.g., $^{15}N/^{14}N$). Absolute abundance ratios are often very small, on the order of a few parts per thousand; thus, isotope values are conveniently expressed relative to a standard and multiplied by 1,000 as "per mil" (mL or ppt) notation, ‰. The final δ value ("del value") is expressed as the amount of the rarer to more common (heavy to light) isotope in the sample, with higher values indicating more of the heavier isotope. By definition, standards have a δ value of 0‰. A positive δ value thus indicates that the sample contains more of the heavy isotope than the standard; a negative δ value indicates that the sample contains less than the standard (Dawson et al. 2002).

As examples, the atmospheric emission of ammonia in animal wastes has an extremely negative del value (–20 mL^{-1}). In contrast, the N (e.g., nitrate) from animal manure is very high (δ values of +20 to +30 mL^{-1}). Groundwater sampled near cattle operations has δ values of +10 to +20 mL^{-1}, usually > +15 mL^{-1}. Other common "signature ranges" for δ values are 0 to +2.0 mL^{-1} for fertilizers, +6.0 to +9.0 for nonpoint N sources, +11 to +13 for point N sources, and +25 for farm wastes (Heaton 1986). The oxygen isotopic value ($^{18}O/^{16}O$) of nitrate can be used to differentiate between fertilizer NO_3^- precipitated from the atmosphere and manure NO_3^- produced by bacterial processes (Wassenaar 1995). The $^{18}O/^{16}O$ of NO_3^- is controlled by the oxygen source. Fertilizers have signatures similar to atmospheric O_2; bacterial-produced NO_3^- represents a combination of groundwater oxygen + atmospheric O_2. The $^{18}O/^{16}O$ value also can reliably indicate denitrification processes, which can also cause variation in ^{15}N enrichments. Sources and biogeochemical processes (e.g., nitrification, denitrification) can be assessed by analyzing both isotopes of nitrate (^{15}N, ^{18}O). Primary producers (phytoplankton, benthic algae, higher plants) and consumers fractionate carbon and nitrogen in predictable ways that allow for the identification of the isotopic composition of their food resources (Cloern et al. 2002). Thus, stable isotope ratios can be used to infer the major source of N taken up by phytoplankton—whether from rainwater, cropland or lawn fertilizer, animal wastes, or human sewage. This ratio approach is limited ideally to analysis of one to two pollution sources near a waterway; with increasing distance from the pollution source, other sources can interfere or "swamp" the signal of interest.

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Molecular Techniques: Microbial Indicators of Pollution

The advent of polymerase chain reaction (PCR) assays and other molecular techniques within the past two decades has significantly advanced rapid detection of pathogenic bacteria, viruses, and protozoans in environmental samples (see the excellent review by Rochelle and Schwab 2006), including some microorganisms that can be used as source indicators of pollution. Fecal bacteria coincide with nutrient enrichment. Several techniques using *Escherichia coli* bacteria have emerged to differentiate human vs. animal pollution sources, including ribotyping, coliphage analysis, and multiple antibiotic resistance. Major advances have occurred, for example, in the use of coliphages (viruses that infect *E. coli*) as reliable indicators of human versus animal sources of fecal waste.

Coliphages generally have longer survivorship under adverse conditions than fecal coliforms (Armon and Kott 1996). Pollutant source tracking has been based mostly on detecting the presence of four groups (I, II, III, and IV) of F-specific RNA (FRNA) coliphages of the family Leviviridae (Stewart et al. 2006). These groups have been confirmed by serological methods and nucleotide sequencing (hybridization with oligonucleotide probes) and physical–chemical data. Group II and III FRNA coliphages have generally (but not always) been associated with human wastes; group I and IV FRNA coliphages have mostly been found in human wastes. Efforts are under way to use additional molecular techniques to develop reliable indicators of poultry, swine, cattle, wildlife, and other major sources of animal wastes (Stewart et al. 2006).

Most molecular methods rely upon nucleic acid sequences and primers and/or probes, so these assays can be highly specific and extremely sensitive, with the potential to detect the presence of a single cell or virus particle of interest. Multiple genes can be targeted for a specific microbial indicator to strengthen the confidence in positive results (Rochelle and Schwab 2006). In addition, many samples can be processed rapidly and simultaneously with automated instrumentation that is comparable in cost to various routinely used equipment in water quality laboratories. Direct enumeration of targeted microbes of interest is not possible, but quantitative techniques calibrated with direct enumeration have rapidly advanced.

Molecular techniques historically have required extensive sample processing and purification, and attempts to analyze environmental samples have met with problems of sample inhibition or partial inhibition that can lead to false negatives and inaccurate quantification of microorganisms of interest. Extensive research has been directed to develop improved high-throughput, cost-effective techniques with minimal inhibition. Other desired attributes are minimal training for use, and reproducibility from user to user (Rochelle and Schwab 2006). Like other techniques, molecular methods should be rigorously evaluated and optimized by users to ensure reliable results (USEPA 2004). Although these methods are not yet in routine use by many water utilities, they provide a suite of tools to support conventional microbiological techniques, and they hold great promise for widespread use once the techniques can be standardized (Rochelle and Schwab 2006).

High-throughput Analysis of Cyanotoxins

The term *toxic cyanobacteria* refers to species that include some populations that are capable of toxin production, that is, *potentially* toxic species (Burkholder 2009). High intraspecific variability in toxin production is well known in cyanobacteria as in eukaryotic "toxic algae"; populations (called strains) within a species commonly range from benign (with negligible toxin production) to highly toxic. Toxicity often varies among cells collected from the same sample in the same bloom, and even among clones of the same isolate.

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For most potentially toxic algae, the genes that "turn on" toxin production remain unknown. Although the factors that regulate expression of toxicity are poorly understood for many cyanobacteria, progress is rapidly advancing through molecular techniques, both in the development of species-specific molecular probes that allow rapid detection of potentially toxic species and in determining the genes that control the synthesis (production) of some cyanotoxins. Of interest to water supply managers in the United States, toxin biosynthesis genes have been discovered for microcystins and cylindrospermopsin (see the excellent review by Neilan et al. 2007). This information has already enabled the genetic screening of source water samples for drinking water quality management. An understanding of the environmental regulation of these toxin biosynthesis genes may provide powerful insights, as well, in controlling toxic bloom events (Neilan et al. 2007).

Toxin biosynthesis pathways in cyanobacteria, as presently understood for hepatotoxins and cytotoxins, seem to mainly involve polyketide synthases (PKSs) and nonribosomal peptide synthetases (PSs). Microcystin synthesis (*mcy*) gene clusters have been sequenced and partially characterized in several genera (*Microcystis*, *Anabaena*, *Planktothrix*; Neilan et al. 2007). Molecular probes have been designed for rapid, specific detection of these genes, and microcystin-specific primers are now available to detect cyanobacteria strains that are capable of synthesizing microcystin toxins. Scientists are also beginning to be able to use quantitative molecular techniques to quantify the toxic microcystin-producing strains versus nontoxic strains present in a given water sample. High N and P have been experimentally correlated with high microcystin production; other influential factors include light trace metals (especially iron) and temperature. Cylindrospermopsin synthetase (from *Cylindrospermopsis raciborskii* and *Anabaena bergii*) has been elucidated as well, and involves three genes—an amidinotransferase (*aoaA*) adjacent to a hybrid NRPS/PKS (*aoaB*) and a type I PKS (*aoaC*—thus far only partially sequenced). The environmental factors controlling cylindrospermopsin production are poorly known; based on one published experimental study, nitrogen-limited cultures of *C. raciborskii* had lowest growth rates and highest toxin production, nitrate-enriched cultures were intermediate, and ammonia-enriched cultures had highest growth rates and lowest toxin production (Saker and Neilan 2001).

Knowledge of the chemical structures of many cyanotoxins has enabled development of high-throughput, rapid-screening analytic methods such as enzyme-linked immunosorbent assays (ELISAs—available for cyanobacterial hepatotoxins and saxitoxins), protein phosphatase inhibition assays (PPIAs—available for microcystins), high-performance liquid chromatography (HPLC), and liquid chromatography (see the excellent review by Sivonen 2007). In addition, molecular techniques have made it possible to discern toxic from nontoxic strains of cyanobacteria in natural water samples (Figure 14-5). High-throughput quantitative molecular techniques such as quantitative polymerase chain reaction (qPCR—SyberGreen® and TaqMan® methods), also known as real-time PCR, have been used to characterize the abundance of toxic versus nontoxic strains of some microcystin-producing cyanobacteria (Sivonen 2007). For example, Rinta-Kanto et al. (2005) used qPCR techniques to determine cell abundance in terms of the total number of cyanobacterial genomes, total number of *Microcystis* genomes, and total number of toxic *Microcystis* genomes present in samples from Lake Erie. They also detected the presence of microcystin producers other than *Microcystis*. DNA chips (microarrays), a developing technology, has the potential to enable the identification of all of the cyanobacteria present in a sample, and also to identify toxin producers depending on the designed probes (Sivonen 2007).

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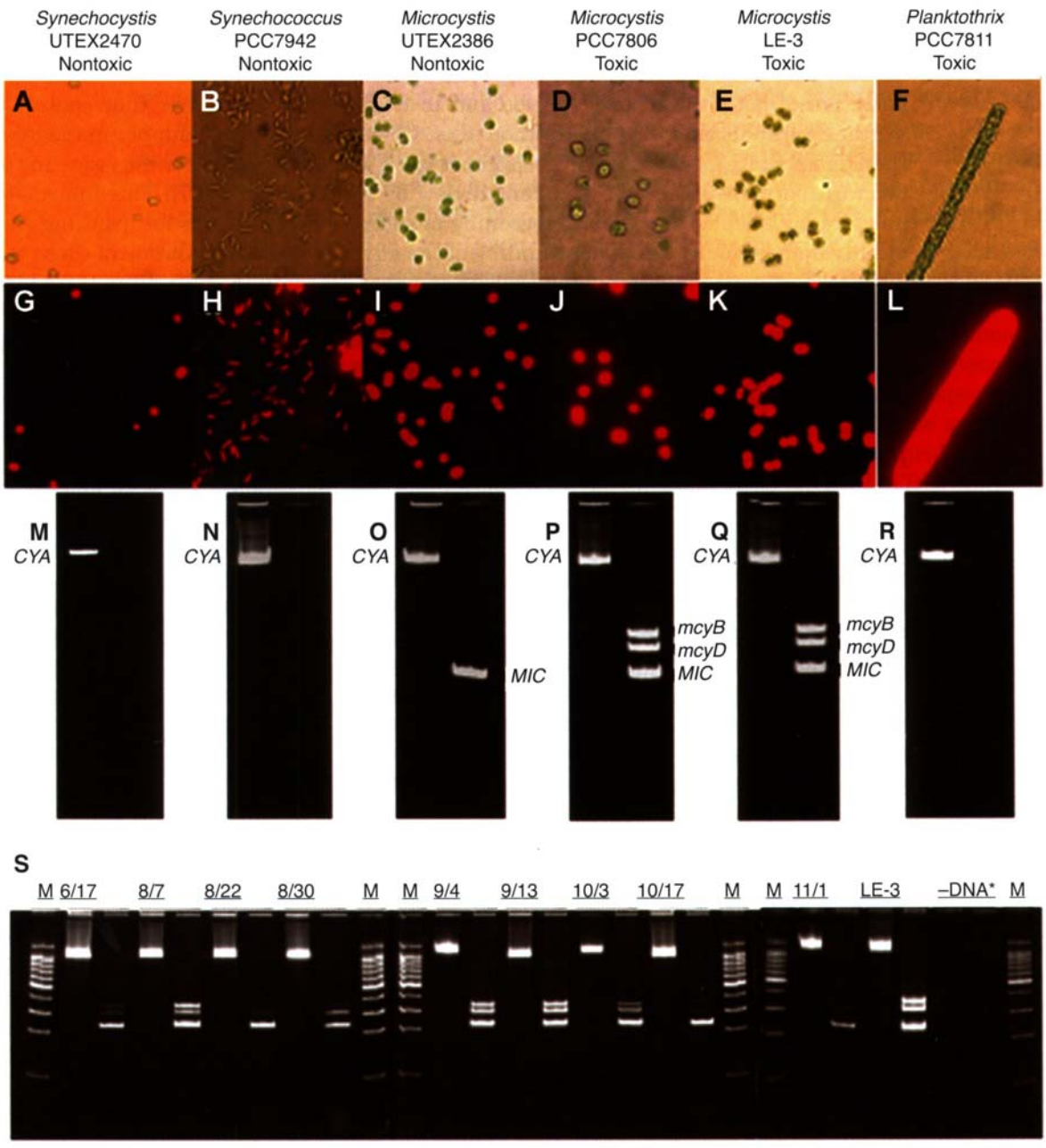

NOTES: (A–F) Detection of *Synechocystis* sp. and *Synechococcus* sp. (both nontoxic), nontoxic *Microcystis* sp., two toxic strains of *Microcystis* sp., and toxic *Planktothrix* sp. using light microscopy. (G–L) Autofluorescence microscopy and (M–R) PCR. (S) Samples from a local pond tested positive for toxic *Microcystis* using PCR and gel electrophoresis (positive control: LE-3 from Lake Erie; negative control, ultrafiltered water from Lake Erie). The presence or absence of appropriately sized PCR bands for cyanobacterial 16S rRNA genes (CYA), *Microcystis* 16S rRNA genes (MIC), and toxin genes *mcyD* and *mcyB* demonstrates the selectivity of the different primers (note: although toxic, *Planktothrix* strain PCC 7811 is not known to produce microcystins). The M in panel (S) denotes 100 base pair DNA markers. The same PCR conditions were applied for the cyanobacterial cultures (M–R) and the environmental samples (S), except that lysed whole cell material was used for the cultures, and genomic DNA was used for the environmental samples.

Figure 14-5 Microscopic and PCR methods for detecting and identifying toxic and nontoxic cyanobacteria (Source: Modified from Ouellette and Wilhelm 2003.)

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Modeling Approaches

Various models have been developed to aid in assessment of the effects of changing watershed land use practices on surface and groundwater quality (e.g., Basnyat et al. 2000, Line et al. 2002, Kelsey et al. 2004, Holland et al. 2004). Airshed models have also advanced in quantifying trends in concentrations, emissions, and deposition of nutrient pollutants (e.g., Walker et al. 2000a, 2000b; Grimm and Lynch 2005), using precipitation chemistry data sets from the National Atmospheric Deposition Program/National Trends Network (2007) and other sources. Groundwater discharge and surface runoff models, in combination with contaminant source loading models, can be applied to assess potential effects of contaminant source areas, and to identify the most susceptible waters for impacts. These models typically use a mass balance approach, which attempts to account for all changes in the mass of a parameter (e.g., P) caused by physical, chemical, and biological processes to predict how a system will respond to land use changes or improved management practices. It has proven difficult, however, to measure many of the relevant hydrologic, geochemical, and biological parameters with any certainty (McCutcheon 1990).

Recently, decadal changes in land use and influences on water quality have been examined with descriptive models such as longitudinal data analysis, as an important first step in the integration of GIS analysis and ecological models (Basnyat et al. 1999). When sufficient monitoring data are available, this empirical approach also provides a powerful tool to address watershed management issues and explore/evaluate management alternatives (McCutcheon 1990). Landscape assessment methods can provide a simple, timely, cost-effective approach for monitoring, targeting, and modeling ecosystem health in watersheds. While most studies have not considered data beyond general land use/land cover grids, GIS analysis has become increasingly valuable in characterizing specific land use practices over time (e.g., Figure 14-6). When combined with longitudinal regression analysis, these powerful tools can elucidate relationships between specific land use practices and water quality.

For example, Rothenberger (2007) completed a decadal analysis of land use influences on water quality in 26 subbasins of the Neuse watershed in North Carolina (e.g., Figure 14-7). Basins or subbasins were delineated using the ArcGIS Arc Hydro software program and elevation (National Elevation Dataset) and hydrographical data (National Hydrography Dataset) from the US Geological Survey. Using the Spatial Analyst extension of ArcGIS 9.1 and the USEPA (1999) land use–land cover (LULC) as the "clip" layer, LULC data sets for differing periods (e.g., 1992, 1996, 2001) were clipped to the dimensions of 26 subbasins for comparison of land uses in the data sets over time. GIS analysis was used together with longitudinal regression analysis to identify specific land use characteristics that most influenced water quality over the decadal period. The LULC grids were imported as separate layers in a GIS interface, and the land use classification system for each data set was then modified to include the land use categories. Once the grids were reclassified, the Spatial Analyst "tabulate area" function was used to calculate the area of each land class within the basin for each year. Latitudes and longitudes for wastewater treatment plants (WWTPs), smaller "package" sewage treatment plants, confined animal feed operations (CAFOs), and sewage sludge land application sites were imported from Microsoft Excel spreadsheets into ArcMap and converted to GIS point files. The numbers of WWTPs, package plants, CAFOs, and sewage sludge land application sites in each subbasin were then quantified and, since subbasins varied in size, expressed as a density (number of points divided by the total area [km^2] of the subbasin).

Prior to running multiple regression analyses, general relationships among all dependent and independent variables were assessed by calculating a Pearson

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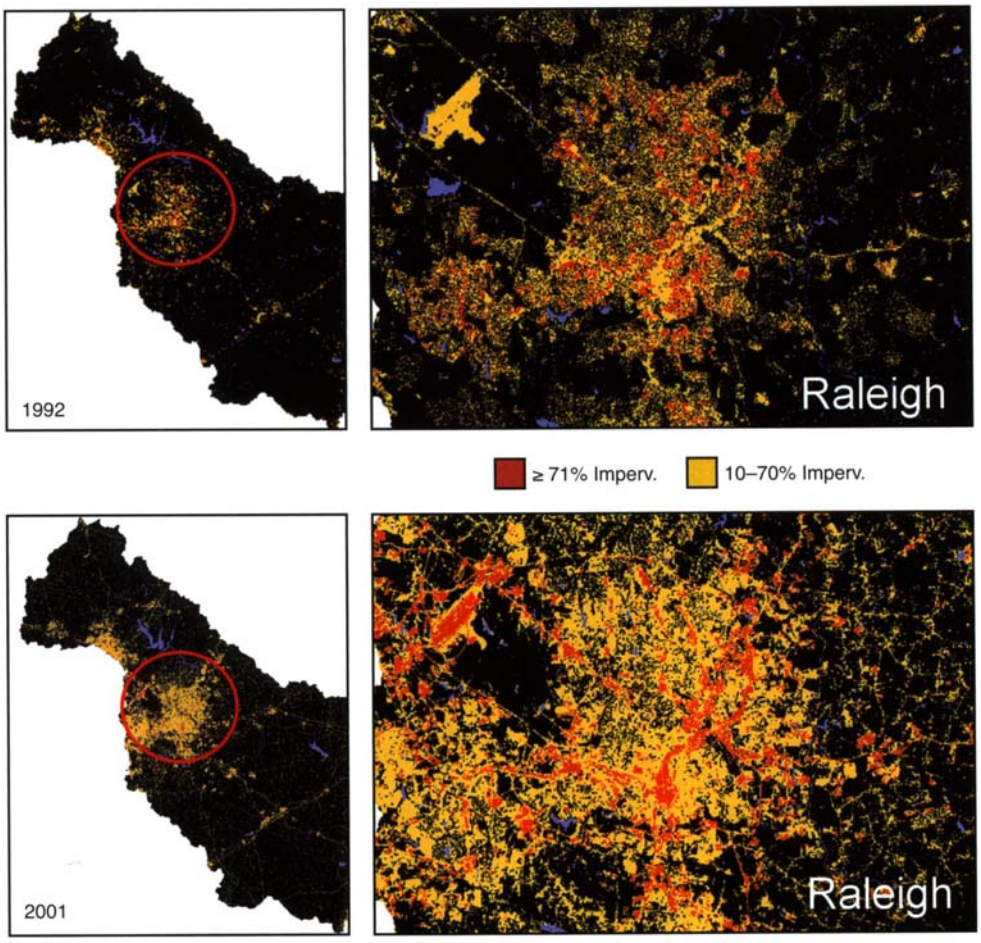

Figure 14-6 Comparison of high- and low-density urban land cover (>70% impervious and 10%–70% impervious, respectively; areas in red circles enlarged) in the upper Neuse River watershed between 1992 and 2001. There was a ~240% increase in total urban cover over the decadal period, including significant encroachment of development in the watershed of the major potable water supply for the area. (Source: Rothenberger 2007.)

correlation coefficient (r) for each possible pair of numeric variables (PROC CORR; SAS Institute Inc. 2006). When independent variables were highly correlated with one another, then only the variable having the stronger correlation (higher r value) with the water quality variable was retained in the model for that variable in order to reduce the confounding effects of multicollinearity (Belsley et al. 1980). Longitudinal regression analysis (PROC MIXED; SAS Institute 2006), with site as a random effect and a first-order autoregressive (AR1) error structure to account for repeated measures over time, was used to determine the direction and magnitude of the relationship between individual water quality variables and land use parameters (Littell et al. 2002). Separate analyses were carried out for each of the dependent variables total phosphorus (TP), soluble reactive phosphate (SRP), nitrate + nitrite (designated as NO_3^-), ammonium (NH_4^+), and total Kjeldahl nitrogen (TKN), each of which was log-transformed prior to analysis. Independent variables were LULC (urban, agricultural, forested, wetland cover), WWTPs, and swine densities in each subbasin. Precipitation data from the N.C. State Climate Office were incorporated into the regression analysis as an independent variable because rainfall events are important in transporting pollutants and nutrients.

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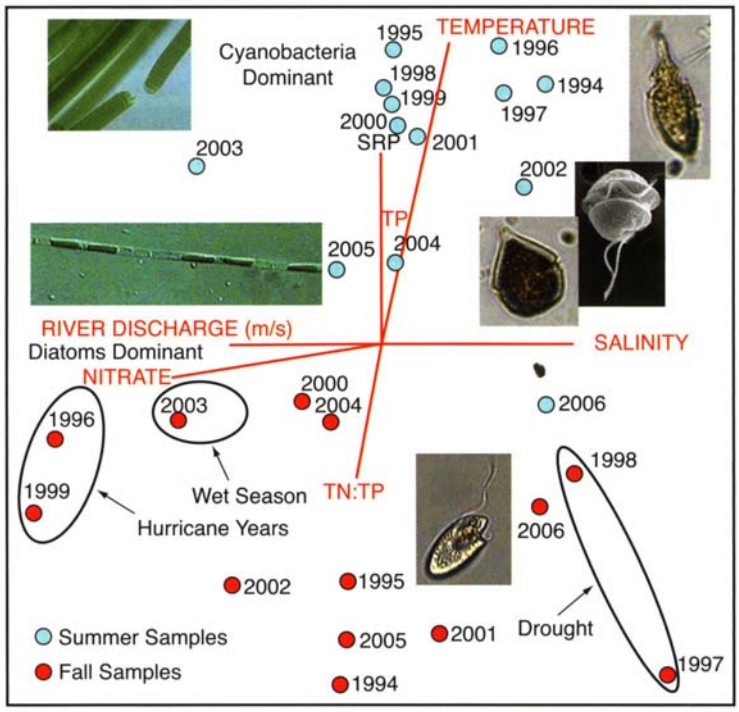

Figure 14-7 Example of nonmetric multidimensional scaling ordination of phytoplankton data, showing that river discharge emerged as an important factor regulating shifts among diatom and dinoflagellate species dominance. Vectors represent the strength of the species gradients. (Source: Rothenberger 2007.)

Analyses indicated that TP concentrations in the Neuse watershed were significantly higher during summer in subbasins with high densities of WWTPs and confined swine feed operations. Nitrate concentrations were significantly higher during winter in subbasins with high numbers of WWTPs, and organic nitrogen was significantly higher in subbasins with higher agricultural coverage, especially with high coverage of pastures fertilized with animal manure. The regression analyses also provided predictive capability of changing water quality conditions with changing land use: As examples, TP concentrations are estimated to increase by 363 $\cdot \log(\mu g\ P\ L^{-1})$ for every additional major WWTP per 100 km^2 ($p < 0.0001$), and by 12 $\cdot \log(\mu g\ P\ L^{-1})$ for every additional minor WWTP per 100 km^2. TKN is estimated to increase by 219 $\cdot \log(\mu g\ TKN\ L^{-1})$ for every additional major WWTP per 100 km^2, and by 5 $\cdot \log(\mu g\ TKN\ L^{-1})$ for every 1% increase in agricultural land cover. TKN was also estimated to decrease by 4 $\cdot \log(\mu g\ TKN\ L^{-1})$ for every 1% increase in forested land cover. The analysis indicated the continuing importance of point as well as nonpoint sources of nutrient pollution: over the decadal period, wastewater discharges in the upper, increasingly urbanized Neuse basin and intensive swine agriculture in the lower basin have been the highest contributors of nitrogen and phosphorus to surface waters.

Ordination Techniques

Long-term (decadal) data sets can also be valuable in assessing phytoplankton responses to chronic eutrophication (nutrient pollution) at the level of harmful or noxious algal groups and species of interest. Ordination techniques such as nonmetric multidimensional scaling (NMDS), indicator species analysis (Dufrêne and Legendre 1997), and BIO-ENV can be used to investigate potential environmental predictors of changes in phytoplankton assemblages under chronic eutrophication.

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Ordination techniques order samples along axes expressing the main trends or gradients in the data. NMDS, considered the most effective, powerful ordination method for ecological data (McCune and Grace 2002), can be used to establish seasonal and year-to-year differences in phytoplankton assemblage structure and to interpret those differences in terms of environmental conditions (e.g., Figure 14-7). NMDS is carried out using PC-ORD (version 4.0) software in the "slow and thorough" autopilot mode using a Sørenson distance matrix. In the resulting NMDS ordination diagrams, the relative distance between the samples reflects similarity in species composition; the closer two taxa are represented in space, the more frequently they co-occur in samples. After samples are arranged in space according to their similarity (or dissimilarity) in species composition, vectors representing environmental variables are superimposed on the ordination diagram to indicate the direction of maximum increase of the variables. BIO-ENV uses the weighted Spearman rank correlation (ρ_s) to correlate the biotic pattern (represented in the phytoplankton station similarity index) with the resemblances (Bray-Curtis distances) between stations computed for each environmental parameter separately (k = 1) and for all possible combinations (k = 2, 3, ..., n) (Clarke and Ainsworth 1993, R Development Core Team 2006). It is then possible to identify the environmental parameter or suite of parameters that correlates best with—and, by implication, may most strongly influence—phytoplankton species and assemblage groups (Rothenberger 2007). For example, in one study, ordination analyses indicated that increasing ammonium concentrations from swine CAFOs and other sources have been an important influence in stimulating potentially toxic phytoplankton species under chronic eutrophication (Rothenberger 2007).

MITIGATIVE AND CONTROL STRATEGIES

Source water protection is the most proactive, effective approach to ensure the highest-quality source water and minimize or prevent algal overgrowth and harmful algal blooms. The frequency and severity of algal blooms can be reduced with good management practices to minimize nutrient inputs to water supplies, including controlled watershed land use to minimize development, impervious surface area and urban/stormwater runoff near water supplies, reduced fertilizer use, reduction or elimination of sewage inputs, appropriate maintenance of septic systems, and use of catch basins and buffer strips to retain nutrient pollution. Reservoir management strategies can also be important, such as minimizing motorboat traffic that could resuspend nutrients from nutrient-rich bottom sediments, and more rapid flushing when possible. Various best management practices (BMPs) to reduce nutrient pollution are available from university extension offices, the US Department of Agriculture, and environmental state agencies.

Once a water source becomes prone to blooms of undesirable algae, various mitigative measures can be applied. The degree of success depends on factors such as the size and natural or imposed flushing and mixing rates in the affected area, the depth and volume of the waterway, the algal species involved, internal nutrient loading characteristics, and the external nutrient loading reductions attained (Cooke et al. 2005).

Noxious and Toxic Algal Blooms

Physical, chemical (herbicide), and biological techniques used in attempts to control harmful algae are limited in rivers, reservoirs, and lakes used as water supplies, and have not substantially improved over the past several decades (Burkholder 2009; Table 14-3). For example, short-term management efforts for cyanobacterial blooms continue to include, where feasible, use of mechanical mixing or bubbling to disrupt

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Table 14-3 Management actions that have been used to reduce (mitigate or "control") algal blooms in waterways, largely compiled from USEPA (1990), Ross and Lembi (1999), McComas (2003), and Cooke et al. (2005)

Aeration (artificial circulation): Can be used to mix part of the water column or the entire water column depending on the water body. Typically, bottom water is lifted to the lake surface where it becomes aerated by oxygen diffusing in from the overlying air. The mixing disrupts dependable pressure gradients in temperature-stratified water bodies, and the increased turbulence discourages algal blooms, especially cyanobacteria. Aeration to mix the entire water column can also prevent severe oxygen depletion from death and decay of an algal bloom; moreover, the increased oxygen reduces phosphorus dissolution from the lake or reservoir sediment into the overlying water column. Aeration of the dark bottom waters can also increase refuge habitats for herbaceous zooplankton to escape fish predators. For best results, aeration should be used before algal blooms can develop; in addition, sufficient aeration must be applied to re-oxygenate the bottom water, or else nutrient-rich bottom water may be circulated to the well-lighted surface waters to stimulate algal blooms.

Biocontrol: Use of various biological agents to control algal growth; the most successful example is the use of grass carp (*Ctenopharyngodon idella*) to control charophytes and, to some extent, filamentous algae,* and the use of silver carp (*Hypophthalmichthys molitrix*) to control filamentous algae.*

Biomanipulation: Addition of predaceous fish to reduce populations of fish that consume herbivorous zooplankton, and/or creation of refuge habitats for herbaceous zooplankton, to increase algal grazing pressure. The complexities of aquatic food webs often makes the desired level of biomanipulation difficult to achieve or maintain; in addition, biomanipulation is generally impractical in large water bodies. If attempted, biomanipulation should be used together with reduction of phosphorus inputs; otherwise, zooplankton effects will be "swamped" by nutrient stimulation, and algal blooms will persist or increase.

Bioscaping: Planting more rooted aquatic vegetation, so that the vegetation and algal colonizers effectively compete with potential phytoplankton bloom formers for nutrients and light. It is recommended that aquatic plant dominance over phytoplankton in mesotrophic (moderately enriched) lakes can be achieved if the aquatic plants cover ~40% or more of the lake bottom, and in moderately P-enriched systems (McComas 2003).

Coagulants: Addition of lime, alum (aluminum sulfate) or ferric chloride have been used, where practicable, to bind with suspended algal cells and then settle out to the bottom of the water body. Alum along with calcium creates buffered alum, which forms a nontoxic precipitate that scavenges phosphorus as it is released from lake sediments. Expensive; one dose can effectively depress algal blooms for several years in lakes with high internal phosphorus loading but minimal loading from external sources. In some controlled settings, certain types of clay can be used to adsorb and remove cyanobacteria from the water column (see review in Burkholder 1992). However, the amount of clay that must be added for this approach to be effective (generally ~35 mg L^{-1} or more) can be detrimental to beneficial aquatic life. At present there are no reliable guidelines or specification for use of clay as a "natural" coagulant to help control cyanobacterial blooms.

Herbicides: A wide array of algicides are commercially available. Caution is warranted in applying herbicides to recreational or drinking water sources, or waterways containing sensitive fish species. This approach can be highly effective in the short term, but is expensive (although, in the short term, often less expensive than aeration or alum) and typically requires repeated applications. Herbicides should be applied when cyanobacteria growth is first detected, *before* blooms begin to develop. Otherwise, the herbicides will cause cyanobacterial cells to lyse and release substantial toxin to the water. Extracellular cyanotoxins are much more difficult for water treatment plant operators to remove. Copper sulfate has been used to kill cyanobacteria since the early 1900s and is marginally effective on other algae such as filamentous greens and charaleans; best results have been achieved in lakes with low alkalinity. Copper can also kill fish and can accumulate to undesirable levels in lake or reservoir sediments. Other common algicides: copper chelates (e.g., Cutrine-Plus®, Algae Pro, K-TEA), diquat (Reward), endothal (potassium salt form, Hydrothol 191®), fluridone (Sonar†), and glyphosate (Rodeo). Herbicides are often recommended as a last resort rather than for routine maintenance.

Table continued next page

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Table 14-3 Management actions that have been used to reduce (mitigate or "control") algal blooms in waterways, largely compiled from USEPA (1990), Ross and Lembi (1999), McComas (2003), and Cooke et al. (2005) (continued)

Increased flushing: Strategy used in reservoirs, where practicable. The decreased water residence time discourages algal blooms.

Liquid dyes: Aquashade and other commercially available compounds can be added to small water bodies to discourage algal blooms by reducing available light.

Physical removal: Use of various netting, raking, vacuuming, or microscreen techniques to physically remove mats of filamentous algae are sometimes practicable albeit labor-intensive. Centrifugal filtration ("swirl removal") devices have also been used to remove cyanobacteria surface accumulations (McComas 2003).

*Including cyanobacteria as well as green algae.
†Trademark of SePRO Corporation.

water-column pressure gradients, or more rapid flushing where feasible; algicides (especially copper sulfate, used for the past several decades); nutrient reductions and increased N:P ratios; and biological manipulations of fish populations to try to increase the abundance of herbivorous zooplankton. Reductions of both N_i and P (N and P co-management), while often economically and politically difficult to achieve, are considered the most effective long-term approach. Algicides or grass carp (*Ctenopharyngodon idella*) continue to be most frequently used, where feasible, to combat nuisance filamentous algae. Of five biocides tested on the mat-forming diatom *D. geminata*, chelated copper may work best if fish health is not compromised (Spaulding and Elwell 2007).

Drinking Water

The multibarrier concept is the most important guiding principle used by most potable water treatment plants to provide safe water (Fox et al. 2006). The present-day reality in North America is that increasing watershed development by urbanization, industrialized agriculture, and other sources of pollution are forcing many communities to rely upon increasingly degraded sources for their water supplies. Thus, the need for multiple protective barriers, mitigative and control measures, and multistep treatments to remove contaminants is ever more critical. In addition to source water assessment (above), the following "barriers" are considered essential strategies:

- *Water treatment plant processes*—such as media filtration, activated carbon filters, enhanced coagulation processes, membrane systems, and disinfection processes.
- *Distribution systems*—maintenance of transmission mains and storage facilities to prevent water quality degradation from noxious and harmful algal blooms.
- *Education*—of water utility personnel, management, policy makers, and the general citizenry about general as well as local issues that could lead to compromised drinking water quality from noxious and harmful algae, and the critical importance of maintaining safe drinking water.

It should be noted that uncovered finished water reservoirs (open reservoirs) are sometimes an important component of a potable water treatment system. A central database is not available for cyanobacterial growths and other algal problems in open reservoirs, but problems reported with microbial contaminants such as *Cryptosporidium* have led the USEPA to require covering such systems or further treating (e.g., rechlorinating) the water as necessary (www.epa.gov/safewater/disinfection/stage2/compliance_idse.html).

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Cyanobacterial toxins. This section draws heavily from the excellent synthesis by Westrick (2007). The Safe Drinking Water Act (SDWA) as amended in 1996 (USEPA 1996) requires the USEPA to publish and update a Contaminant Candidate List (CCL, thus far 1998 and 2005) of microorganisms and substances that are known to occur or anticipated to occur in public water systems, are not yet subject to proposed or promulgated National Primary Drinking Water Regulations, and may require regulation under the SDWA. Thus, the CCL is the source of future drinking water regulations in the United States, and the USEPA uses the CCL to prioritize research efforts to aid in assessment of whether contaminants should be regulated. At present, there are no drinking water standards specifically for cyanobacteria and their toxins, but since 1998 the CCL has included cyanobacteria (blue-green algae), other freshwater algae, and their toxins as contaminants (see www.epa.gov/safewater/ccl/). Cyanobacteria are also among USEPA's unregulated contaminants for which monitoring is required. In 2001, USEPA identified several cyanobacterial toxins as high priorities for potential health risks in source and finished waters of drinking water utilities in the United States. USEPA's National Center for Environmental Assessment then completed a toxicological review of several common microcystins (class of closely related cyclic hepatotoxic peptides), selected for focus from among ~80 known (Zurawell et al. 2005, USEPA 2006a). A draft toxicological review on cylindrospermopsin is available (USEPA 2006b), and a toxicological review of anatoxin-a is planned.

The World Health Organization (WHO 2003) has set 1 μg microcystin-LR L^{-1} in drinking water as a guideline for human health protection. The guideline is based on one common cyanotoxin, despite the fact that multiple toxins often are present in affected waters, because supporting animal and human toxicity data are incomplete for most other cyanotoxins. The most common routes of human exposure to cyanotoxins are by direct contact with toxic blooms, ingestion of contaminated water, or aerosol inhalation. Acute health effects (e.g., through water contact sports) have included eye and ear irritation, mouth ulcers, nausea, vomiting, diarrhea, fever, and cold or flu symptoms. A density of 100,000 toxic *Microcystis* cells mL^{-1} (estimated to correspond to ~50 μg chlorophyll *a* L^{-1} and 100–200 μg microcystins L^{-1}) was recommended by WHO as a guideline for a moderate health alert in recreational waters. Such guidelines can be misleading, however, because cyanotoxin production depends on the cyanobacterial species, nutrient supplies, and other environmental conditions. Phytoplankton chlorophyll *a* levels, or total cyanobacterial abundance—even cell densities or biomass of known potentially toxic species—often poorly predict the toxicity of blooms (Figure 14-8). In addition, surface and shoreline accumulations or "scums" potentially can create severe health hazards for humans, wildlife, and domestic animals.

Research on removal or inactivation of cyanotoxins by various procedures in drinking water utilities thus far is limited to only a few toxins (Tables 14-4 through 14-6). Cyanotoxins may be intracellular or extracellular (inside or outside the cells, respectively). Many cyanobacteria retain their toxins inside the cells until the cells become senescent and "leaky" or are stressed and lysed by other factors such as ill-timed application of herbicides during established blooms. The effectiveness of removal/inactivation processes depends on the type, concentration, and location (intracellular/extracellular) of the toxins. Intracellular toxins are more easily removed because conventional treatment processes can be used, such as flocculation, coagulation, sedimentation, filtration, and chlorination. New approaches such as pretreatment with oxidants other than chlorine, ballasted flocculation, and activated carbon have also been effective to varying degrees. Extracellular toxins can sometimes be effectively oxidized (inactivated) by various disinfectants; otherwise, advanced treatment procedures must be used, such as membrane filtration, ultraviolet (UV) photolysis, granular activated

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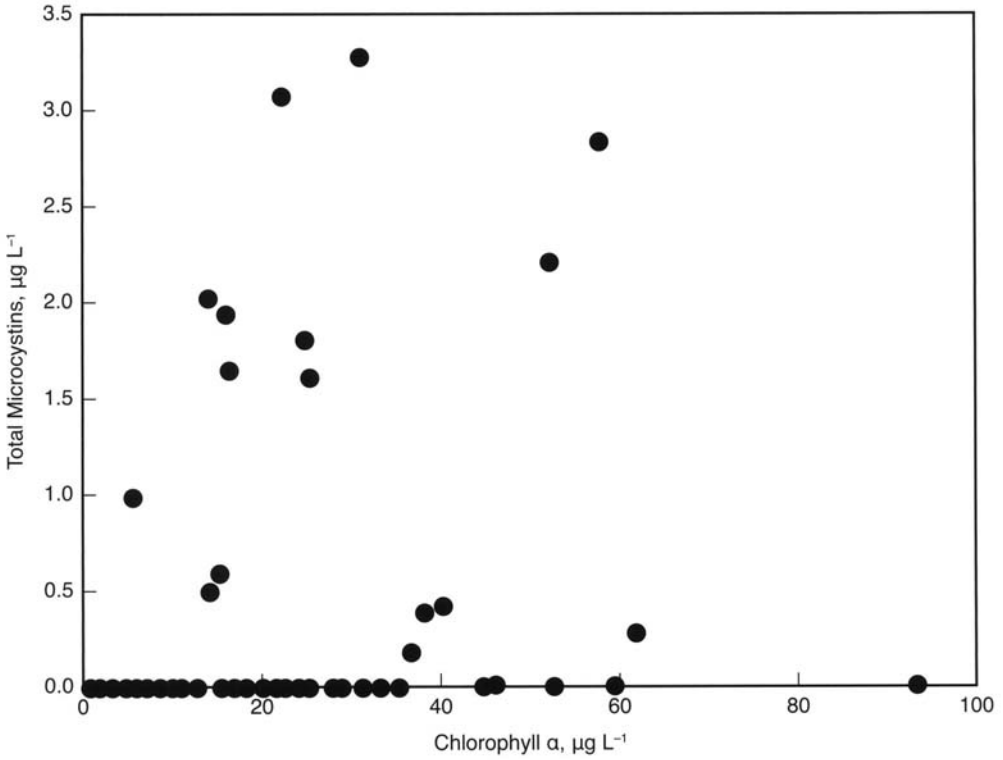

Figure 14-8 Relationship between microcystins and chlorophyll *a* concentrations in 58 lakes and reservoirs located in New York state, USA (R^2 = 0.1, n = 140). Chlorophyll *a* would have been a poor predictor of bloom toxicity, at least considering total microcystin cyanotoxins. (Source: Boyer, G.L. (2007, *Lake and Reservoir Management*), with permission from the North American Lake Management Society.)

carbon (GAC) absorbers, and ozone (Westrick 2007). Most available information is on microcystin-LR and several other microcystins, with more limited data for anatoxin-a, saxitoxins, and cylindrospermopsin.

Disinfectant effectiveness is often considered as C · T or CT, where C = the disinfectant concentration (in mg L^{-1}) and T = the contact time (in minutes). The lower the CT value, the more effective the disinfectant at inactivation. Effectiveness is strongly influenced by the water temperature and pH, the concentration(s) of other (interfering) compounds present that can react with the disinfectant, and the target organism or toxin (Westrick 2007). For example, cyanotoxins are differentially susceptible to chlorine oxidation. Chlorine is not effective at inactivating anatoxin-a, microcystins and cylindrospermopsin are more effectively activated at lower pH values (e.g., Tables 14-4, 14-5), and saxitoxins are more effectively inactivated at higher pH values. In one study, for example, saxitoxins (saxitoxin STX; C2-saxitoxin; decarbamoylated saxitoxin dcSTX; and gonyautoxins GTX2, GTX3, and GTX5) were degraded by 90% at pH 9 (Nicholson et al. 2003). Chlorine has also been shown to effectively degrade cylindrospermopsin (CT 15 mg L^{-1} min^{-1}) at pH 6 over a 30-minute contact time when the free chlorine residual is >0.5 mg L^{-1} (Senogles et al. 2000). The potential has not been tested for chronic toxicity of chlorination by-products of cyanotoxin degradation. Based on acute toxicity tests (mouse bioassays, PPIAs, and mutagenicity), toxicity was not detected from chlorination by-products of microcystins and saxitoxins, but chlorination by-products of cylindrospermopsin caused some liver damage and genotoxicity (reviewed in Westrick 2007). Among other disinfectants, chloramines and chlorine dioxide thus far have been ineffective in degrading microcystins at cost-effective

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Table 14-4 Chlorine CT values (C·T, where C = the disinfectant concentration in mg L^{-1} and T = the contact time in min) for reducing microcystin concentration to 1 μg L^{-1} for a batch reactor (Source: Acero et al. 2005, reprinted from Westrick 2007, with permission)

pH	MC-LR (μg L^{-1})	CT values (mg L^{-1} min^{-1})			
		10°C	15°C	20°C	25°C
6	50	46.6	40.2	34.8	30.3
	10	27.4	23.6	20.5	17.8
7	50	67.7	58.4	50.6	44.0
	10	39.8	34.4	29.8	25.9
8	50	187.2	161.3	139.8	121.8
	10	110.3	94.9	82.3	71.7
9	50	617.2	526.0	458.6	399.1
	10	363.3	306.6	269.8	234.9

dosages (Kull et al. 2004, Newcombe and Nicholson 2004), and other cyanotoxins have not been tested. Ozone has been shown to effectively degrade microcystins and anatoxin-a, but has been relatively ineffective in degrading saxitoxins (Table 14-5).

UV energy has been used effectively at cost-effective levels (<100 mJ cm^{-2}) to inactivate many microbial pathogens. Unfortunately, this approach has not been shown to be economically feasible for cyanotoxins, which require much higher UV doses for degradation (Table 14-5). Further research may establish treatment combinations involving UV that can effectively inactivate cyanotoxins.

Powdered or granular activated carbon (PAC and GAC, respectively), especially mesoporous (pore size 2–50 nm) wood-based activated carbon, appear to be effective in removing extracellular cyanotoxins (Table 14-5). It should be noted, however, that the efficiency of activated carbon should be expected to vary from site to site depending on the background total dissolved organic carbon concentrations, cyanobacteria abundance, the occurrence of taste and odor compounds and agricultural chemicals, and other factors. Both PAC and GAC filters are commonly used to remove various toxins and taste and odor compounds. GAC filters can be used alone or with other filter media, and are replaced or reactivated when 70%–80% of the total organic carbon (TOC) in the prefiltered water is retained in the filtrate. Cyanotoxins have been shown to vary in adsorption efficiency. For example, among tested microcystins, adsorption to PAC varied as RR > YR > LR > LA (Cook and Newcombe 2002); among saxitoxins, adsorption was highest for STX (most toxic) > GTX > C (Newcombe and Nicholson 2004).

Among other types of filters, reverse osmosis and nanofiltration membranes (pore size 100 Daltons and 200 Daltons, respectively) have been shown to remove many pathogens and large molecules above the membrane pore size (AWWA 2007). Their effectiveness at cyanotoxin removal depends in part on the cyanotoxin concentrations, but the available data thus far are promising (Table 14-5). Ultrafilters (pore size 0.22 μm) and microfilters (pore size > 0.22 μm) are generally effective at removing intact cyanobacterial cells and their associated intracellular toxins, with minimal cell breakage.

Taste and odor compounds. A wide array of freshwater planktonic and benthic algae, including numerous cyanobacteria and ochrophytes (Ochrophyta: some diatoms, chrysophytes, synurophytes, and tribophytes), but also cryptophytes (*Cryptomonas rostratiformis*), dinoflagellates (*Peridinium* sp., *Gymnodinium* sp.), and chlorophytes (e.g., *Scenedesmus subspicatus* and *Chlorella*, *Eudorina*, *Chlamydomonas*, *Gonium*, *Volvox*, *Chara* spp.) produce cucumberlike, fishy, rancid, oily, or "skunklike" odorous

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Table 14-5 Treatment of drinking water to remove or inactivate cyanotoxins, compiled from Knappe et al. (2004), Newcombe and Nicholson (2004), Svrcek and Smith (2004), and Westrick (2007) and references therein

Treatment	Effectiveness at Cyanotoxin Degradation
Oxidants	
Chlorine	Effectively inactivates extracellular microcystins, cylindrospermopsin (pH 6–8); inactivates saxitoxins (pH ≥9). By-products from inactivation of microcystins, and saxitoxins are not acutely toxic; by-products from inactivation of cylindrospermopsin are acutely toxic (cylindrospermopsin); chronic toxicity tests of by-products are still lacking.
Chloramines, chlorine dioxide	Ineffective in degrading microcystins at cost-effective dosages.
Ozone	Effectively inactivates extracellular microcystin and anatoxin-a when residual ozone is maintained for several minutes at moderate temperatures (16°–22°C; CT 0.1 $\mu g\ L^{-1}\ min^{-1}$ and 0.3 $\mu g\ L^{-1}\ min^{-1}$, respectively). By-products of microcystin degradation are not toxic in acute toxicity tests. Degrades saxitoxins with low to moderate effectiveness.
Electrochemical degradation	New, advanced oxidation procedure (hydroxyl radical mechanism): at an applied current of 100 mA, the degradation rate was 0.219 min^{-1} and the half-life was 2.5 min—potentially promising future approach.
UV Treatment (λ 200–300 nm)	Not economically feasible: for example, 1,530 to 20,000 mJ cm^{-2} is needed to degrade extracellular microcystins, cylindrospermopsin, and anatoxin-a.
Activated carbon	Effectively removes cyanotoxins depending on the concentration and other factors.
Powdered and granular activated carbon (PAC, GAC) filters	Extracellular microcystin adsorption, considering four types: microcystin-RR > microcystin-YR > microcystin-LR > microcystin-LA. Extracellular adsorption of several types of saxitoxins: STX (saxitoxins, most toxic) > GTX (gonyautoxin) > C-saxitoxins.
Other Filters	
RO, nanofilters	Reverse osmosis (RO) can remove >95% of extracellular microcystin-LR, (100–200 Daltons) microcystin-RR (10–130 $\mu g\ L^{-1}$); nanofilters can remove ~80%–100% of extracellular microcystin.
Ultrafilters (0.22 μm)	Effectively remove intact cyanobacterial cells and their intracellular toxins.
Microfilters (>0.22 μm)	Effectively remove larger intact cyanobacterial cells and their intracellular toxins.
Conventional	Coagulation and sedimentation followed by filtration effectively removes intact cyanobacterial cells and their intracellular toxins.

compounds (Burkholder 2009, AWWA 2003). Taste and odor producing species often occur within a mixed algal assemblage that includes many benign taxa (nonproducers). The approach generally taken is to remove their undesirable compounds during water treatment. Aesthetic problems such as taste and odor, while a major concern to water treatment plant operators and consumers, are considered by the USEPA under National Secondary Drinking Water Regulations because they are not a safety issue. These nonmandatory regulations are meant to provide general guidelines to water managers for taste, color, and odor (www.epa.gov /safewater/consumer/2ndstandards.html).

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Table 14-6 Some recent studies on cyanotoxin removal in raw and finished water at drinking water utilities

Utilities	Findings
10 (North America)	Source water: 80% (539 samples) positive for microcystins (ELISA test); 4.3% >1 μg L^{-1} (World Health Organization guideline). Finished water: only 2 samples >1 μg L^{-1} (Carmichael 2001).
5 (Wisconsin)	Source water: ~0.1–7 μg L^{-1}. Finished water: the plants demonstrated 4 on 1 lake, 1–3 log removal of microcystin. Pretreatment alone (i.e., prior to filtration) with potassium permanganate, copper sulfate, and powdered activated carbon (PAC) reduced cyanotoxins by 61% (Karner et al. 2001).
2 (Lake Zurich at depth 30 m; and the Wahnbachtal Dam, Germany)	First plant with preozonation (1.0 mg L^{-1}), rapid sand filtration, intermediate ozonation (0.5 mg L^{-1}), GAC filtration, and slow sand filtration—removed 8.0 μg microcystins L^{-1} to <1 μg L^{-1}. Second plant used conventional treatment (flocculation/sedimentation, sand filtration, and chlorination of pipeline) reduced 2.0 μg microcystins L^{-1} to <1 μg L^{-1} (Hoeger et al. 2005).
5 (Oklahoma, Vermont, Texas, California, Florida)	Source waters with 0.2–0.5 μg microcystins L^{-1}; finished waters all ≤0.05 μg L^{-1}. Typical conventional treatment removed 2–5 log algal units mL^{-1}; finished water generally had <2 algal units (solitary cells, colonies, or filaments) mL^{-1} (S. Wood and colleagues, 2005, in Westrick 2007).
Various (Florida)	Microcystins: Source waters with ~4–12.5 μg L^{-1}. Of 15 paired raw/finished water samples, toxin was undetectable in 8 finished water samples. Of the remainder, the median concentration was ~0.9 μg/L (range ~0.1 to 9.9 μg/L) (Burns 2005, 2007).
	Anatoxin-a: Source waters were mostly <1 μg L^{-1}. Of 36 paired samples, finished water was below detection in 7 samples, 19 samples had <1 μg L^{-1}, and 10 samples had 1–9 μg L^{-1} (Burns 2005).
	Cylindrospermopsin (150 paired samples): Source waters contained <1 μg L^{-1} (~12%), 1–10 μg L^{-1} (~27%), and >10–100 μg L^{-1} (61%). Finished water contained undetectable toxin (42%), <1 μg L^{-1} (3%), 1–10 μg L^{-1} (10%), and >10 μg L^{-1} (45%) (Burns 2005).
8 (North Carolina)	Source waters (72 samples) with <0.7 μg microcystins L^{-1}; finished water samples all had <0.05 μg L^{-1}, except for 3 samples from one water treatment plant which had 0.05–0.06 μg L^{-1} (Touchette et al. 2007, and J. Burkholder 2007, North Carolina State University, unpublished data).

Most taste and odor incidents impart "earthy," "musty," or "muddy" odors and have been related to geosmin and 2-methylisoborneol (MIB) produced primarily by planktonic and benthic cyanobacteria including some potentially toxic species (Figure 14-9). Geosmin and MIB can taint water even if present in concentrations as low as a few nanograms per liter (ng L^{-1}) (Wnorowski 1992). Other odorous substances from algae can include organic sulfur compounds, hydrogen sulfide, and various volatile organic compounds (VOCs) (Watson 2004). Many algal VOCs (AVOCs) have been identified, some of which are also produced by bacteria or fungi. Relatively few of these compounds—certain terpenoids, sulfur compounds, and PUFA derivatives—cause most algal-associated taste and odor problems. High variability in AVOC production occurs both among species and among strains within a given species in similar environmental conditions.

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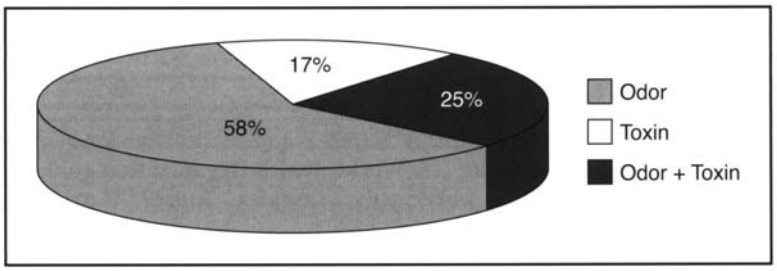

Figure 14-9 Survey of 45 cyanobacterial species for odor (geosmin, 2-methylisoborneol, or ß-cyclocitral) and cyanotoxin production (microcystins and/or neurotoxins) (Source: Watson 2004, *Journal of Toxicology and Environmental Health*, with permission)

Methods of detection and identification of taste and odor substances include sensory analysis and instrumental analysis by gas chromatography/mass spectrometry. Conventional treatment (coagulation/sedimentation/chlorination) generally has failed to destroy geosmin, MIB, and many other odorous compounds. Therefore, treatment methods most often used include activated carbon adsorption, ozone, chlorine dioxide, peroxone, and potassium permanganate, but oxidation by-products may also have undesirable odors. Geosmin and MIB are variably removed by activated carbon. Chlorine has been found to be effective at removing odors from low-level inorganics such as hydrogen sulfide, but the technique generally requires air stripping or dechlorination. Chlorine is also effective with organic sulfides, disulfides, and mercaptans, but it can enhance odors from geosmin and MIB. Potassium permanganate has been effective as well, but if too much is added, manganese dioxide forms and blackens the water. Biological degradation (by some bacteria and yeasts) of odorous organic compounds can be an attractive alternative to chemical–physical treatment.

Distribution system challenges. One of the biggest challenges confronting water utilities is to maintain quality water supplies throughout the distribution system. This problem repeatedly has been emphasized in considerations about distribution systems for finished drinking water (see Fox et al. 2006 and references therein). Water quality degrades over distance through loss of disinfectant residual, temperature changes, flow velocity changes, biofilm sloughing, sediment suspension within the pipes, pipe breaks and cracks, intrusions of contaminants from pressure changes and cross-connections, regrowth of bacteria that survived the treatment processes as resistant forms, and growth of bacteria as biofilms on pipe walls and surfaces in storage reservoirs and tanks (Fox et al. 2006). In a somewhat analogous situation, noxious and harmful algae can form thick biofilms that contaminate and even clog channelized rivers used as water supplies; drainage ditches that are critical for cropland agriculture; and open irrigation and drinking water distribution canals, for example, in many areas of the western United States (Figure 14-10).

Although noxious and harmful algae commonly affect open distribution systems in the United States and throughout the world, their economic effects are not tracked in any centralized database and, thus, their quantitative impacts are unknown except for isolated local events (Burkholder 2009). For example, in the Salt River Project Canal that supplies water and electricity to Phoenix and Tempe, Ariz., control of aquatic weeds, primarily *Cladophora*, has been a major operations/maintenance task since the 1980s (Lembi et al. 1988), costing well over $1 million per year. Since the 1980s in California alone, the US Bureau of Reclamation has used at least 3,000 metric tons of copper sulfate per year for algal control in canals.

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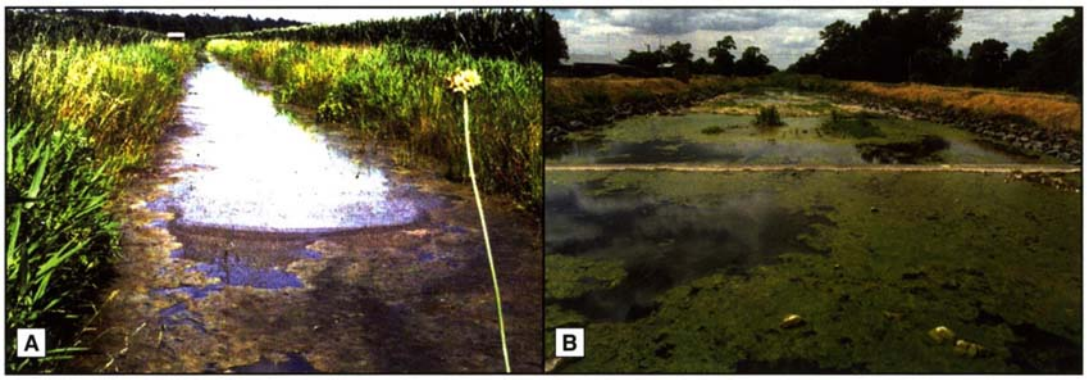

Figure 14-10 Examples of algal overgrowth in conduit drainage ditches and channelized river segments: (A) Cyanobacteria bloom in a drainage ditch from agricultural fields in the southeastern United States (Courtesy of R. Evans, North Carolina State University, with permission). (B) Mats of cyanobacteria and filamentous green algae in a channelized stream in the Walla Walla, Wash., district of the US Army Corps of Engineers (Courtesy of US Army Corps of Engineers, with permission).

The noxious diatom *D. geminata* can remain viable in damp conditions for weeks, and its spread has been related to recreational fishing activities as well as irrigation, water diversions, and waterfowl hunting. In the United States it has expanded from western states through the Southeast. Its benthic matlike growths can blanket up to 200 km of length in affected streams for several months each year, with up to 100% coverage several centimeters thick. Massive brownish, slimy growths of *D. geminata* frequently have been mistaken for raw sewage. In North America and Europe, blooms commonly occur in rivers below impoundments characterized by stable flows and more constant temperatures. *D. geminata* also thrives in canal systems in the western United States that transport water for human consumption, agriculture, and hydroelectric power. In some systems, managers are already attempting to regularly remove *D. geminata*.

FUTURE DIRECTIONS

While significant progress has been made in the past decade to assess and combat nutrient pollution, algal blooms, and their toxins and taste and odor compounds, the following examples highlight some of the many frontiers that remain. High-quality long-term data sets with at least weekly to biweekly frequency of data collection at multiple stations are needed to more accurately estimate nutrient loadings to source waters (Burkholder et al. 2006). The ability to predict the effects of increasing watershed development on surface and groundwater quality depends on understanding the relationship between landscape properties and these nutrient loading patterns. Rothenberger's (2007) decadal analysis of land use changes and effects on surface water quality represents one of the few available studies that examine relationships between land cover and both N and P over a decadal period, including land use data beyond general LULC grids. Descriptive models such as this longitudinal analysis should be developed for watersheds of interest and used together with predictive models to more accurately describe past and present relationships between land use and water quality, as well as providing projections of future relationships. These models should integrate watershed land use/water quality changes with effects of climate change, for example, elevated temperature, and carbon dioxide, which can stimulate potentially toxic cyanobacteria (Whitton and Potts 2000, Burkholder 2009).

The effects of nutrient enrichment and other environmental factors on the growth and toxicity of noxious and harmful algae are complex and often vary among species

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and among strains within a given species. Ordination and other advanced analyses should be used to advance understanding and predictive capability about the responses of noxious and harmful algae to chronic eutrophication, and also to gain insights about recovery of source waters in cases where nutrient reductions can be achieved. Improved strategies to reduce algal toxins and taste and odor compounds in source waters will also require strengthened understanding of the environmental, physiological, and molecular controls on the production of these substances.

Improved miniaturized biosensors such as dual spectral radiometers are being developed to assess cyanobacterial abundance (Ritchie and Zimba 2005) in source surface waters. Technologies for image analysis of algal cells are also being improved and may be amenable in the future for coupled use with RTRM to track *in situ* cyanobacterial populations of interest. For example, the FlowCAM®, a continuous imaging flow cytometer, is designed to characterize particles in the microplankton size range (20–200 μm), and can automatically record information on the size and fluorescence of cells (Sieracki et al. 1998). The water sample of interest is pumped through a thin glass chamber in the instrument, illuminated by a green laser. A video camera captures an image of each object (cell) that passes through the field of view under a 10× objective microscope lens, and images can be further magnified on the computer screen for visual identification (Buskey and Hyatt 2006). Software enables comparison of each captured image to a set of up to eight reference images of a targeted species for identification, based on a computerized pixel-based comparison of the cell in the sample versus the reference images for the species of interest. At present, this technology works best for species of distinct shape and size but, as yet, is not as reliable in discerning organisms that closely resemble the target species (Buskey and Hyatt 2006).

Most available information on effective treatment to remove cyanotoxins in drinking water treatment has focused on several microcystins. Basic studies are needed to develop new and/or improved methods for eliminating anatoxin-a, saxitoxins, cylindrospermopsin, and their derivatives in water treatment processes. Molecular methods to detect potentially toxic cyanobacteria and their toxins (PCR + DNA chips) are rapidly advancing. Thus far they have emphasized microcystin producers and should be developed, as well, for species that produce other toxins. These techniques eventually will provide powerful monitoring tools, and also a way to finally examine toxic cyanobacterial populations in their natural environment so that much more specific, quantitative information can be gained about the interacting environmental factors that promote highly toxic blooms. Techniques for toxin analysis should be streamlined and standardized for general use, including provision of cyanotoxin standards through a safe clearinghouse (de la Cruz et al. 2007).

Toxicity-based monitoring systems targeting at least several common cyanotoxins within a given ecosystem, rather than traditional chlorophyll- or cell count–based monitoring, are still in development phase. Such programs would significantly advance assessment of the potential risks for drinking water supplies and recreational activities from specific cyanobacterial blooms (Boyer 2007). Ideally they would involve molecular techniques to detect the presence of genes involved in toxin synthesis as a rapid, routine, cost-effective initial screening method for toxic strains. Research is also ongoing to create cyanotoxin microarrays that can be linked to real-time automated monitoring systems. This technology would provide a powerful early warning system to strengthen protection of potable water supplies and public health.

Finally, centralized national and international databases are needed for information on noxious and harmful freshwater algal blooms. These databases should include information on the location, species, environmental conditions, bloom magnitude and duration, identification/quantities of toxins and taste and odor compounds, source water treatment, open distribution system treatment, and the costs of treatment and

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other economic effects. The insights gained would help the general citizenry and policy makers to understand the economic impacts of noxious and harmful algal blooms, the formidable challenges they represent for water utility and natural resource managers, and the need by water utilities to provide the resources to meet these expanding challenges. Data-mined information on environmental conditions, toxins, taste and odor, and algal/other microbial diversity could also strengthen insights about the causes and potential for improved control of these blooms.

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REFERENCES

Acero, J.L., E. Rodriguez, and J. Meriluoto. 2005. Kinetics of reaction between chlorine and the cyanobacterial toxin microcystins. *Water Res.*, 39:1628–1638.

American Water Works Association (AWWA). 2003. Manual M7, *Problem organisms in water: Identification and treatment.* 3rd ed. Denver, Colo.: AWWA.

American Water Works Association (AWWA). 2007. *Manual M46, Reverse osmosis and nanofiltration.* 2nd ed. Denver, Colo.: AWWA.

Armon, R., and Y. Kott. 1996. Bacteriophages as indicators of pollution. *Crit. Rev. Environ. Sci. Technol.*, 26:299–335.

Basnyat, P., L.D. Teeter, K.M. Flynn, and B.G. Lockaby. 1999. Relationship between landscape characteristics and nonpoint source pollution inputs to coastal estuaries. *Environ. Manage.*, 23:539–549.

Basnyat, P., L.D. Teeter, B.G. Lockaby, and K. Flynn. 2000. The use of remote sensing and GIS in watershed level analyses of non-point source pollution problems. *For. Ecol. Manage.*, 128:65–73.

Belsley, D.A., E. Kuh, and R.E. Welsch. 1980. *Regression diagnostics: Identifying data and sources of collinearity.* New York: Wiley.

Boyer, G.L. 2007. The occurrence of cyanobacterial toxins in New York lakes: Lessons from the MERHAB-Lower Great Lakes Program. *Lake Reservoir Manage.*, 23:153–160

Burkholder, J.M. 1992. Phytoplankton and episodic suspended sediment loading: Phosphate partitioning and mechanisms for survival. *Limnol. Oceanogr.*, 37:974–988.

Burkholder, J.M. 2009. Harmful algae. In *Encyclopedia of inland waters.* G.E. Likens, ed. New York: Elsevier.

Burkholder, J.M., D.A. Dickey, C. Kinder, R.E. Reed, M.A. Mallin, G. Melia, M.R. McIver, L.B. Cahoon, C. Brownie, N. Deamer, J. Springer, H. Glasgow, D. Toms, and J. Smith. 2006. Comprehensive trend analysis of nutrients and related variables in a large eutrophic estuary: A decadal study of anthropogenic and climatic influences. *Limnol. Oceanogr.*, 51:463–487.

Burns, J., Jr. 2005. Cyanobacteria: The Florida experience. Presentation at the International Symposium on Cyanobacterial Harmful Algal Blooms, Research Triangle Park, N.C. (September).

Burns, J. 2007. Toxic cyanobacteria in Florida waters. In *Proceedings of the interagency, international symposium on cyanobacterial harmful algal blooms (ISOC-HAB): State of the science and research needs.* H.K. Hudnell, ed. New York: Springer.

Buskey, E.J., and C.J. Hyatt. 2006. Use of the FlowCAM for semi-automated recognition and enumeration of red tide cells (*Karenia brevis*) in natural plankton samples. *Harmful Algae*, 5:685–692.

Carmichael, W.W. 2001. *Assessment of blue-green algal toxins in raw and finished drinking water.* Denver, Colo.: Awwa Research Foundation.

Chorus, I., and J. Bartram, eds. 1999. *Toxic cyanobacteria in water—A guide to their public health consequences, monitoring and management.* New York: E&FN Spon.

Clarke, K.R., and M. Ainsworth. 1993. A method of linking multivariate community structure to environmental variables. *Mar. Ecol. Prog. Ser.*, 92:205–219.

Cloern, J.E., E.A. Canuel, and D. Harris. 2002. Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Bay estuarine system. *Limnol. Oceanogr.*, 47:713–729.

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Cook, D., and G. Newcombe. 2002. Removal of microcystin variants with powdered activated carbon. *Water Sci. Technol.: Water Supply*, 2(5-6):201–207.

Cooke, G.D., E.B. Welch, S.A. Peterson, and S.A. Nichols. 2005. *Restoration and management of lakes and reservoirs*. 3rd ed. Boca Raton, Fla.: CRC Press.

Dawson, T.E., S. Mambelli, A.H. Plamboeck, P.H. Templer, and K.P. Tu. 2002. Stable isotopes in plant ecology. *Ann. Rev. Ecol. System.*, 33:507–559.

de la Cruz, A.A., P. Rublee, J.M Hungerford, P.V. Zimba, S.W. Wilhelm, J.A.O. Meriluoto, K. Echols, M.T. Meyer, G. Stelma, R. Mandeville, L. Lawton, K. Sivonen, and A. Furey. 2007. Analytical methods workgroup report. In *Proceedings of the interagency, international symposium on cyanobacterial harmful algal blooms (ISOC-HAB): State of the science and research needs*. H.K. Hudnell, ed. New York: Springer.

Dufrêne, M., and P. Legendre. 1997. Species assemblages and indicator species: The need for a flexible asymmetrical approach. *Ecol. Mono.*, 67:345–366.

Fox, K.R., D.J. Reasoner, and R. Gertig. 2006. Water quality in source water, treatment, and distribution systems. In *Manual M48, Waterborne pathogens*. 2nd ed. Denver, Colo.: AWWA.

Glasgow, H.B., Jr., J.M. Burkholder, R.E. Reed, A.J. Lewitus, and J.E. Kleinman. 2004. Real-time remote monitoring of water quality: A review of current applications, and advancements in sensor, telemetry, and computing technologies. *Jour. Exper. Mar. Biol. Ecol.*, 300:409–448.

Grimm, J.W., and J.A. Lynch. 2005. Improved daily precipitation nitrate and ammonium concentration models for the Chesapeake Bay watershed. *Environ. Pollut.*, 135:445–455.

Heaton, T.H.E. 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: A review. *Chem. Geol.*, 59:87–102.

Hecky, R.E., and P. Kilham. 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment. *Limnol. Oceanogr.*, 33:796–822.

Hoeger, S.J., B.C. Hitzfield, and D.R. Dietrich. 2005. Occurrence and elimination of cyanobacterial toxins in drinking water treatment plants. *Toxicol. Appl. Pharmacol.*, 203:231–242.

Holland, A.F., D.M. Sanger, C.P. Gawle, S.B. Lerberg, M.S. Santiago, G.H.M. Riekerk, L.E. Zimmerman, and G.I. Scott. 2004. Linkages between tidal creek ecosystems and the landscape and demographic attributes of their watersheds. *Jour. Exper. Mar. Biol. Ecol.*, 298:151–178.

Jüttner, F. 1995. Physiology and biochemistry of odorous compounds from freshwater cyanobacteria and algae. *Water Sci. Technol.*, 31:69–78.

Kalff, J. 2002. *Limnology*. Upper Saddle River, N.J.: Prentice Hall.

Karner, D.A., J.H. Standridge, G.W. Harrington, and R.P. Barnum. 2001. Microcystin algal toxins in source and finished drinking water. *Jour. AWWA*, 93:72–81.

Kelsey, H., D.E. Porter, G. Scott, M. Neet, and D. White. 2004. Using geographic information systems and regression analysis to evaluate relationships between land use and fecal coliform bacterial pollution. *Jour. Exper. Mar. Biol. Ecol.*, 298:197–209.

Knappe, D., R.C. Belk, D. Briley, S. Gandy, and N. Rastogi. 2004. *Algae detection and removal strategies for drinking water treatment plants*. Denver, Colo.: Awwa Research Foundation.

Kull, T., P.H. Backlund, K.M. Karlsson, and J.A. Meriluoto. 2004. Oxidation of the cyanobacterial hepatotoxin microcystin-LR by chlorine dioxide: Reaction kinetics, characterization, and toxicity of reaction products. *Environ. Sci. Technol.*, 38:6025–6031.

Lembi, C.A., S.W. O'Neal, and D.F. Spencer. 1988. Algae as weeds: Economic impact, ecology and management alternatives. In *Algae and human affairs*. C.A. Lembi and J.R. Waaland, eds. New York: Cambridge University Press.

Line, D.E., N.M. White, D.L. Osmond, G.D. Jennings, and C.B. Mojonnier. 2002. Pollutant export from various land uses in the Upper Neuse River Basin. *Water Environ. Res.*, 74:100–108.

Littell, R.C., W.W. Stroup, and R.J. Freund. 2002. *SAS for linear models*. 4th ed. Cary, N.C.: SAS Publishing.

McComas, S. 2003. *Lake and pond management guidebook*. New York: Lewis Publishers.

McCune, B., and J.B. Grace. 2002. *Analysis of ecological communities*. Gleneden Beach, Ore.: MjM Software Design.

McCutcheon, S.C. 1990. *Water quality modeling, Vol. I: Transport and surface exchange in rivers*. Boca Raton, Fla.: CRC Press.

Mitsch, W.J., and J.G. Gosselink. 2000. *Wetlands*. 4th ed. New York: John Wiley & Sons.

National Atmospheric Deposition Program/ National Trends Network (NADP/NTN). 2007. *National Atmospheric Deposition*

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Program (NRSP-3)/National Trends Network (chemistry data set). Champaign, Ill.: NADP Program Office, Illinois State Water Survey.

National Research Council. 2000. *Clean coastal waters—Understanding and reducing the effects of nutrient pollution.* Washington, D.C.: National Academy Press.

Neilan, B.A., L.A. Pearson, M.C. Moffitt, K.T. Mihali, M. Kaebernick, R. Kellmann, and F. Potami. 2007. The genetics and genomics of cyanobacterial toxicity. In *Proceedings of the interagency, international symposium on cyanobacterial harmful algal blooms (ISOC-HAB): State of the science and research needs.* H.K. Hudnell, ed. New York: Springer.

Newcombe, G., and B. Nicholson. 2004. Water treatment options for dissolved cyanotoxins. *Jour. Water Supply Technol. Aqua,* 53:227–239.

Nicholson, B.C., G.R. Shaw, J. Morrell, P.J. Senogles, T.A. Woods, C. Papageorgiou, C. Kapralos, W. Wickramasinghe, B.C. Davis, G.K. Eaglesham, and M.R. Moore. 2003. Chlorination for degrading saxitoxins (paralytic shellfish poison) in water. *Environ. Technol.,* 24:1341–1348.

Ouellette, A.J.A., and S.W. Wilhelm. 2003. Toxic cyanobacteria: The evolving molecular toolbox. *Front. Ecol. Environ.,* 1:359–366.

R Development Core Team. 2006. *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, www.r-project.org/index.html.

Reed, R.E., J.M. Burkholder, and E.H. Allen. 2010. Recent developments in online monitoring technology for surveillance of algal blooms, potential toxicity, and physical–chemical structure in rivers, reservoirs, and lakes. In *Manual M57, Algae: Source to treatment.* Denver, Colo.: AWWA.

Ritchie, J.C., and P.V. Zimba. 2005. Hydrological application of remote sensing water quality, including sediment and algae. In *Encyclopedia of hydrology.* M. Anderson, ed. Chichester, UK: John Wiley.

Rinta-Kanto, J.M., A.J.A. Ouellette, G.L. Boyer, M.R. Twiss, T.B. Bridgeman, and S.W. Wilhelm. 2005. Quantification of toxic *Microcystis* spp. during the 2003 and 2004 blooms in western Lake Erie. *Environ. Sci. Technol.,* 39:4198–4205.

Rochelle, P.A., and K.J. Schwab. 2006. Molecular detection of waterborne microorganisms. In *Manual M48, Waterborne pathogens.* 2nd ed. Denver, Colo.: AWWA.

Ross, M.A., and C.A. Lembi. 1999. *Applied weed science.* 2nd ed. Upper Saddle River, N.J.: Prentice Hall.

Rothenberger, M.B. 2007. Long-term impacts of changing land-use practices on water quality and phytoplankton assemblages in the Neuse Estuary ecosystem, North Carolina. Ph.D. diss. North Carolina State University, Raleigh.

Saker, M.L., and B.A. Neilan. 2001. Varied diazotrophies, morphologies, and toxicities of genetically similar isolates of *Cylindrospermopsis raciborskii* (Nostocales, Cyanophyceae) from northern Australia. *Appl. Environ. Microbiol.,* 67:1839–1845.

SAS Institute, Inc. 2006. *SAS/STAT 9.1 User's guide,* ver. 8. Cary, N.C.: SAS Institute.

Schindler, D.W. 1977. Evolution of phosphorus limitation in lakes. *Science,* 196:260–262.

Senogles, P., G. Shaw, M. Smith, R. Norris, R. Chiswell, J. Mueller, R. Sadler, and G. Eaglesham. 2000. Degradation of the cyanobacterial toxin cylindrospermopsin from *Cylindrospermopsis raciborskii,* by chlorination. *Toxicon,* 38:1203–1213.

Sieracki, C.K., M.E. Sieracki, and C.S. Yentsch. 1998. An imaging-inflow system for automated analysis of marine microplankton. *Mar. Ecol. Prog. Ser.,* 168:285–296.

Sivonen, K. 2007. Emerging high throughput analyses of cyanobacterial toxins and toxic cyanobacteria. In *Proceedings of the interagency, international symposium on cyanobacterial harmful algal blooms (ISOC-HAB): State of the science and research needs.* H.K. Hudnell, ed. New York: Springer.

Spaulding, S., and L. Elwell. 2007. *Increase in nuisance blooms and geographic expansion of the freshwater diatom* Didymosphenia geminata: *Recommendations for response.* www.epa.gov/region8/water/didymosphenia/White%20Paper%20Jan%202007.pdf (accessed Nov. 21, 2009).

Stewart, J.R., J. Vinjé, S.J.G. Oudejans, G.I. Scott, and M.D. Sobsey. 2006. Sequence variation among group III F-specific RNA coliphages from water samples and swine lagoons. *Appl. Environ. Microbiol.,* 72:1226–1230.

Svrcek, C., and D.W. Smith. 2004. Cyanobacteria toxins and the current state of knowledge on water treatment options: A review. *Jour. Environ. Eng. Sci.,* 3:155–185.

Touchette, B.W., J.M. Burkholder, E.H. Allen, J.L. Alexander, C.A. Kinder, J. James, and C.H. Britton. 2007. Eutrophication and cyanobacteria blooms in run-of-river

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impoundments in North Carolina, USA. *Lake Reservoir Manage.*, 23:179–192.

US Environmental Protection Agency (USEPA). 1990. *The lake and reservoir restoration guidance manual.* EPA 440/4-90-006. Washington, D.C.: USEPA, Office of Water. Manual and technical supplement available online: http://nepis.epa.gov/EPA/html/Pubs/pubtitleOAR.htm.

US Environmental Protection Agency (USEPA). 1996. Safe Drinking Water Act (SDWA) Amendments of 1996 (P.L. 104-182) for state Source Water Assessment Programs [sections 1453 and 1428(b)] and for Source Water Petition Programs (section 1454). www.epa.gov/OGWDW/sdwa/summ.html.

US Environmental Protection Agency (USEPA). 1997. *State source water assessment and protection programs—Final guidance.* EPA 816-R-97-009. Washington, D.C.: USEPA, Office of Water.

US Environmental Protection Agency (USEPA). 1999. Neuse River Basin land cover land use data. Accessed June 4, 2008: http://www.lib.ncsu.edu/gis/neuslclu.html.

US Environmental Protection Agency (USEPA). 2004. *Quality assurance/quality control guidance for laboratories performing PCR analyses on environmental samples.* EPA 815-B-04-001. Washington, D.C.: USEPA, Office of Water.

US Environmental Protection Agency (USEPA). 2006a. *Toxicological reviews of cyanobacterial toxins: Microcystins LR, RR, YR and LA* (external review draft). EPA/600/R-06/139. Washington, D.C.: USEPA, Office of Water.

US Environmental Protection Agency (USEPA). 2006b. *Toxicological reviews of cyanobacterial toxins: Cylindrospermopsin* (external review draft). EPA/600/R-06/138. Washington, D.C.: USEPA, Office of Water.

Walker, J., D. Nelson, and V.P. Aneja. 2000a. Trends in ammonium concentration in precipitation and atmospheric ammonia emissions at a coastal plain site in North Carolina, USA. *Environ. Sci. Technol.*, 34:3527–3534.

Walker, J.T., V.P. Aneja, and D.A. Dickey. 2000b. Atmospheric transport and wet deposition of ammonium in North Carolina. *Atmos. Environ.*, 34:3407–3418.

Wassenaar, L.I. 1995. Evaluation of the origin and fate of nitrate in the Abbostford Aquifer using the isotopes of ^{15}N and ^{18}O in NO_3^-. *Appl. Geochem.*, 10:391–405.

Watson, S.B. 2004. Aquatic taste and odor: A primary signal of drinking-water integrity. *Jour. Toxicol. Environ. Health*, 67:1779–1795.

Westrick, J.A. 2007. Cyanobacterial toxin removal in drinking water treatment processes and recreational waters. In *Proceedings of the interagency, international symposium on cyanobacterial harmful algal blooms (ISOC-HAB): State of the science and research needs.* H.K. Hudnell, ed. New York: Springer.

Wetzel, R.G. 2001. *Limnology.* 3rd ed. New York: Academic Press.

Whitton, B.A., and M. Potts, eds. 2000. *The ecology of cyanobacteria—Their diversity in time and space.* Boston: Kluwer Academic Publishers.

Wnorowski, A.U. 1992. Tastes and odours in the aquatic environment: A review. *Water S. Afr. Water Sci. Adv.*, 18:203–214.

World Health Organization (WHO). 2003. Algae and cyanobacteria in fresh water. In *Guidelines for safe recreational waters, Vol. 1: Coastal and fresh waters.* Geneva: WHO.

Zurawell, R.W., H. Chen, J.M. Burke, and E.E. Prepas. 2005. Hepatotoxic cyanobacteria: A review of the biological importance of microcystins in freshwater environments. *Jour. Toxicol. Environ. Health Part B*, 8:1–37.

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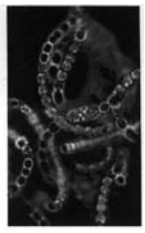

Chapter 15

Algal Taste and Odor

Sue B. Watson

INTRODUCTION

Taste and Odor: Current Management Issues

There is a widespread belief that the frequency and severity of algal and cyanobacterial* impairment of surface waters is increasing, largely because of the degradation of watersheds and their receiving waters. The dissolved and particulate organic material produced by these algal outbreaks has a significant effect on the chemistry of the water, and can have far-reaching ecological and socioeconomic impacts. Volatile compounds released during their growth and decline can include terpenoids, biogenic sulfides, lipid and pigment derivatives, aromatics, long-chain hydrocarbons, amines and other substances, many of which can cause foul taste and odor (T&O) in inland waters.† Traditionally, surface water T&O has been associated with eutrophic waters (e.g., Persson 1982, Jüttner 1984, Wnorowski 1992, Kenefick et al. 1992, Davies et al. 2004), but there has been a recent growth in the number of events reported in meso-oligotrophic and remediated systems, including some of the largest surface waters of the world, the North American Great Lakes (Nicholls 1982, 1995; McBean 1990; Watson et al. 2001a, 2007a).

T&O outbreaks are still not well understood, and hence they are often inadequately controlled by current management approaches. Many odor events are not anticipated and/or untraced, and there is a widespread, disturbing reliance on reactive treatment (Bruchet 1999). But the growing pressure to provide safe, palatable drinking water is highlighting the critical need for a more sustainable, proactive approach, based on the key factors that underlie these events.

* For brevity, eukaryotic algae and cyanobacteria will be collectively referred to as "algae" unless indicated; note that cyanobacteria belong to the bacteria Monera and differ in reproduction, cell structure, and much of their biochemistry from true algae (Chlorophyta, Chrysophyta, Cryptophyta, Dinophyta, Euglenophyta, Haptophyta, etc.).

† The discussion will implicitly refer to inland surface waters as opposed to marine or groundwater sources.

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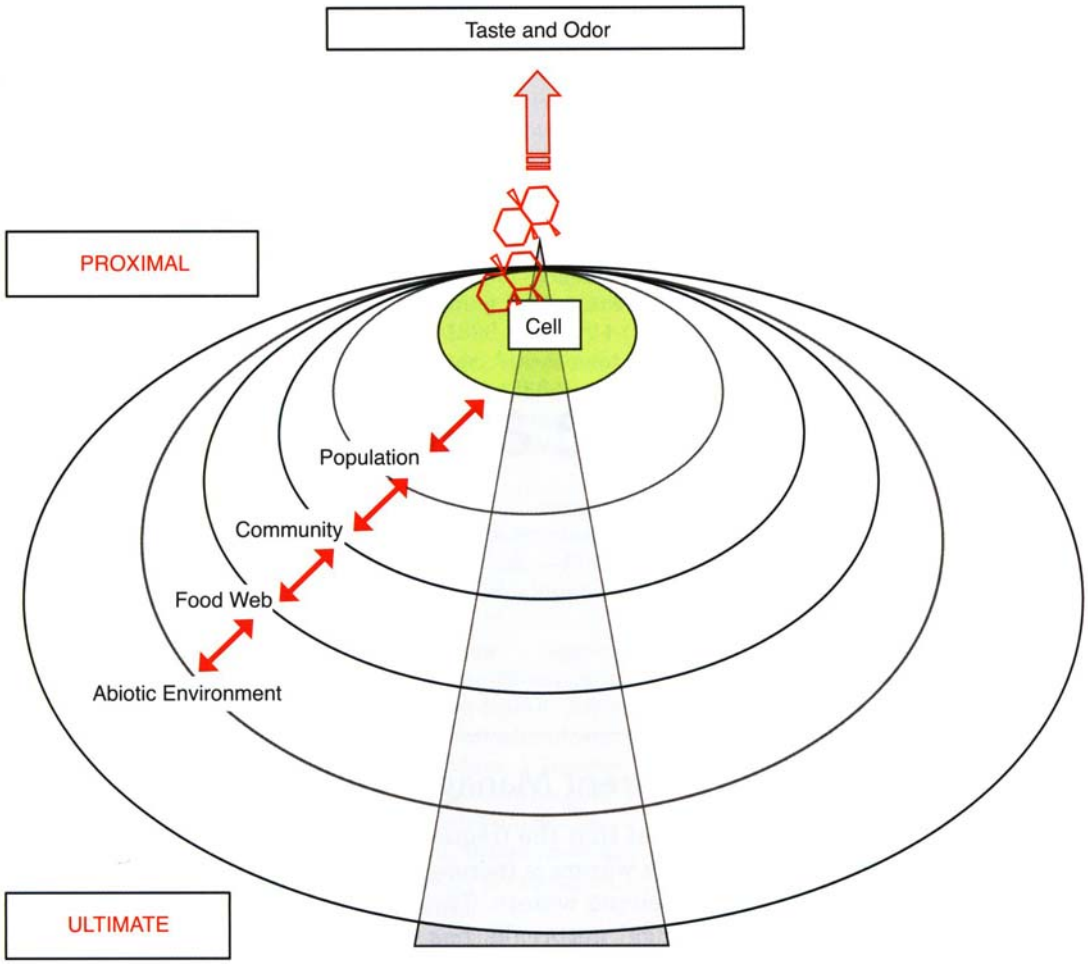

Figure 15-1 Large- and small-scale processes contributing and modifying algal taste and odor production

Major T&O Sources

Successful T&O management requires an integrated understanding of the major physical, chemical, and biological factors operating at different spatial and temporal scales (Figure 15-1). T&O events are caused by one or more volatile organic compounds (VOCs) from one or more of two major types of sources: (1) biological (microbial, algal, plant, animal), (2) human-made/abiotic (mineral, industrial, agricultural/urban, water treatment, distribution pipes, etc.). Identifying the major sources and compounds for each T&O outbreak event presents numerous challenges. This chapter addresses the first category, biological sources, one of the most commonly reported and significant causes of T&O, and focuses on algae and cyanobacteria.

Algal T&O: Spatial-Temporal Scales of Influence

Because of its complexity, algal T&O is widely misunderstood. Algal VOC production and its eventual detection by humans as T&O—for example, in recreational waters, at treatment plants, and in tap water—result from a series of physical, chemical, and biological interactions that operate across a broad spectrum of space and time to cause—or modify—T&O. Successful diagnosis, treatment, and long-term control require an integrated understanding of these processes. This chapter encapsulates

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this by highlighting the key factors operating at (1) microscale (cell and molecular) levels—VOC characteristics, known algal producers (including potential link[s] between T&O and toxin production); (2) population and community levels, where algal species dynamics and nonalgal biota play significant roles in VOC production; and (3) ecosystem (source water) levels, where large-scale processes modify and transport source organisms and their VOCs across space and time. Finally, a brief section is included on detection and monitoring, treatment, and control.

DIAGNOSING THE SPECIES AND THEIR CHEMICALS

At a microscale level, the key challenge is to identify the primary agents, keeping in mind that there may be more than one species and compound involved. Given the chemical and biological "soups" typical of most source waters, this can be a daunting task, particularly because the cells and their VOCs may not be present together in the same sample (as, for example, with attached algal sources). Nevertheless, some important general trends help to narrow down these choices considerably.

VOCs: Natural Sources and Culture/Analytical Artifacts, Chemical "Fingerprints"

A survey of published and unpublished sources yields an extensive list of VOCs attributed to algal origins. Research continually adds to this list, which is likely far from complete (Table 15-1). Note, however, that the majority of these compounds were reported from cultures (and most of these, nonaxenic, i.e., containing bacterial and/or other microorganisms). Fewer than half the compounds have actually been identified in surface waters (with or without the presence of their suspected algal producers). This suggests that a significant number of reported VOCs may represent compounds generated by culture artifacts (e.g., produced under unnatural culture conditions, or from equipment contamination or growth media, etc.), or microbial contaminants (bacteria, fungi, or even other algae). It is important to distinguish these from naturally occurring VOCs (see Jüttner 1995, Rashash et al. 1995, Höckelmann and Jüttner 2004).

Similarly, while VOC production has been reported from individual algal and cyanobacterial species belonging to most of the major algal groups, these represent only a very small fraction of the huge number of known species (which have not been tested), so many potential sources are as yet unidentified.

Table 15-1 also demonstrates that no single algal group or species has a unique "organoleptic fingerprint," in part because many biosynthetic pathways are ubiquitous. All known algal VOCs are produced by several (or many) species, which belong to the same or different taxonomic groups.

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Table 15-1 List of major taste and odor causing algal volatile organic compounds (VOCs), with odor descriptors (where known), known producers listed with their taxonomic groups: CY = Cyanobacteria; GR = Chlorophyta (green algae), CHR = Chrysophyta (Diatoms [Dia], Chrysophyceae [Chr] and Synurophyceae [Syn]), DN = Dinophyta; CR = Cryptophyta, RD = Rhodophyta

			Taxicological Group								
					CHR						
VOC	Reference	Descriptor	CY	GR	Dia	Chr	Syn	DN	CR	RD	Taxa
Amines and Other N-Metabolites											
1-Aminopropane (propylamine)	1		x	x	x	x					*Ankistrodesmus, Microcystis, Ochromonas; Nitzschia* sp.
2-Aminopropane (isopropyl amine)	1	Ammonia				x					*Ochromonas*
Butanolamine	1	Fishy		x							*Chlamydomonas*
Ethanolamine	1	Ammonia				x					*Ochromonas*
Methylamine	1	Rotten fish			x						*Nitzschia*
Trimethylamine	1	Fishy → ammonia				x					*Ochromonas*
Indole (2,3-benzopyrrole)	37	Floral → fecal	x								Cyanobacteria field samples; possibly derived from bacterial fermentation
Skatole (-methyl-2,3-benzopyrrole)	37	Floral → fecal	x								Cyanobacteria field samples; possibly derived from bacterial fermentation
p-Cresol	37	Medicinal	x								Cyanobacteria field samples; possibly from bacterial fermentation
3-Methyl indole	38	Fecal		x							Decaying *Cladophora* mats, derived from bacterial fermentation
Aromatics											
Benzaldehyde	8	Almond					x				*Synura uvella*
Benzyl acetate	7	Jasmine		x							*Chlamydomonas peterfii*
Benzyl alcohol	7	Sharp burning taste, faint aromatic		x							*C. peterfii*

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Table 15-1 List of major taste and odor causing algal volatile organic compounds (VOCs), with odor descriptors (where known), known producers listed with their taxonomic groups: CY = Cyanobacteria; GR = Chlorophyta (green algae), CHR = Chrysophyta (Diatoms [Dia], Chrysophyceae [Chr] and Synurophyceae [Syn]), DN = Dinophyta; CR = Cryptophyta, RD = Rhodophyta (continued)

			Taxicological Group								
					CHR						
VOC	Reference	Descriptor	CY	GR	Dia	Chr	Syn	DN	CR	RD	Taxa
2,6-Dimethyl pyrazine	5	Cocoa, roasted nuts, coffee			x						*Asterionella formosa*
2-Furfural	7	Sweet, cinnamon, caramel, almond	x								*Synura petersenii*
3-Methoxy-2-isopropyl pyrazine	5	Earthy/potato bin			x						*A. formosa*
2-Phenylphenol	7		x								*Anabaena laxa; Oscillatoria* sp.; *O. tenuis; Phormidium* sp.
Phenethyl alcohol (benzyl carbinol)	7	Rose	x	x			x				*C. peterfii; Microcystis aeruginosa; S. petersenii*
Sulfur Compounds											
1,2,4 Trithiolate	4					x					*Ochromonas danica*
4-Methylthio-1,2-dithiolane	16; 17	Pungent		x							*Chara globularis C. hispida; C. baltica; Nitella translucens; Tolypella nidifica*
5-Methylthio,-1,2,3,-trithiane	16, 17	Pungent		x							*C. globularis; C. hispida; C. baltica; N. translucens; T. nidifica*
Dihydrogen sulfide	18	Rotten eggs	x								*Anacystis nidulans; Synechococcus cedrorum; O. chalybea; O. tenuis; Phormidium autumnale; Plectonema boryanum*
Dimethyl disulfide	5, 10, 18, 20, 8, 6	Septic, garlic, putrid	x	x	x	x					*M. aeruginosa, Anabaena* sp., *Poteriochromonas malhamensis*
Dimethyl sulfide	18	Intense, sulfurous	x								*A. nidulans; S. cedrorum; Oscillatoria chalybea; O. tenuis; Phormidium autumnale; Plectonema boryanum; Nostoc*

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Table 15-1 List of major taste and odor causing algal volatile organic compounds (VOCs), with odor descriptors (where known), known producers listed with their taxonomic groups: CY = Cyanobacteria; GR = Chlorophyta (green algae), CHR = Chrysophyta (Diatoms [Dia], Chrysophyceae [Chr] and Synurophyceae [Syn]), DN = Dinophyta; CR = Cryptophyta, RD = Rhodophyta (continued)

			Taxicological Group								
					CHR						
VOC	Reference	Descriptor	CY	GR	Dia	Chr	Syn	DN	CR	RD	Taxa
Dimethyl tetrasulfide	5, 10, 20, 2, 1, 38	Putrid	x	[x]		x	x				*Poteriochromonas malhamensis; Ochromonas danica, Synura petersenii; Oscillatoria* sp., decaying *Cladophora*
Dimethyl trisulfide	5, 10, 19; 21	Septic, garlic, putrid, swampy	x	x	x	x	x				*Poteriochromonas malhamenesis; S. petersenii; Ochromonas danica; Scenedesmus subspicatus; Aulacoseira granulata; Oscillatoria* sp.
Dimethyl pentasulfide	38	Septic, garlic, putrid, swampy		[x]							Decaying *Cladophora* mats, derived from bacterial fermentation
Isopropyl disulfide	15		x								*M. aeruginosa*
Isopropyl methyl disulfide	15		x								*M. aeruginosa*
Isopropyl trisulfide	15		x								*M. aeruginosa*
Isopropyl thiol	15		x								*M. aeruginosa*
Methylethanethiolate	10					x					*Ochromonas danica*
Methylpropanethiolate	10					x					*Ochromonas danica*
Methyl 2-methylpropanethiolate	10					x					*Ochromonas danica*
Methyl 3-methylbutanethiolate	10					x					*Ochromonas danica*
Methylthio propionate	2,6					x					*P. malhamensis*
Methyl mercaptan	18		x								*S. cedrorum*
Methyl propyl disulfide	20	Foul; cooked onion	x								*M. aeruginosa*
Methylthio 3-methylbutanate	2,6					x	x				*P. malhamensis, S. petersenii*
Methylthio acetate	2, 6					x	x				*P. malhamensis, S. petersenii*

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Table 15-1 List of major taste and odor causing algal volatile organic compounds (VOCs), with odor descriptors (where known), known producers listed with their taxonomic groups: CY = Cyanobacteria; GR = Chlorophyta (green algae), CHR = Chrysophyta (Diatoms [Dia], Chrysophyceae [Chr] and Synurophyceae [Syn]), DN = Dinophyta; CR = Cryptophyta, RD = Rhodophyta (continued)

			Taxicological Group								
					CHR						
VOC	Reference	Descriptor	CY	GR	Dia	Chr	Syn	DN	CR	RD	Taxa
Terpenes and Terpenoids											
λ-Cadinene	22		x								*Oscillatoria splendida*
1,2-Dihydro-1,1,6-trimethylnapthalene	9									x	*Cyanidium caldarium*
2,2,6-Trimethyl cyclohexanone	5	Tobacco/ musty		x							*Scenedesmus subspicatus*
2-Methylisoborneol	20, 26, 27	Earthy, musty	x								See Table 15-3
3-Methylbut-2-en-1-ol	22	Alcoholic	x	x	x						*Anabaena cylindrica; Synechococcus PCC6301*
4-Methylpent-3-en-2-one	14		x	x							*Anabaena cylindrica ; Chlorella sorokiana; C. homosphaera*
6-Methyl-5-hepten-2-ol	14	Fruity; esterlike	x								*A. cylindrica*
6-Methyl-5-hepten-2-one	5, 8, 9, 20	Fruity; esterlike	x	x	x	x					*Synechococcus, A. cylindrica; M. aeruginosa; S. subspicatus, A. granulata; S. uvella; C. caldarium*
6-*cis*,2-*trans*-6-but-2-enylidenetrimethylcyclohex-1-ene	9									x	*Cyanidium caldarium*
β-Cyclocitral	5, 7, 8, 14, 20, 28	Tobacco, flowery; smoky/moldy; blackcurrant leaves	x	x		x	x				*Microcystis wesenbergii; M. aeruginosa; M. viridis; Anabaena* sp. *A; cylindrica; Dinobryon cylindricum; S. subspicatus; S. uvella*
Hydroxy-β-cyclocitral	7		x								*M. aeruginosa*
Camphor	5	Aromatic, musty, woody		x							*S. subspicatus*
α-Campholene	5	Earthy/musty			x						*A. formosa*

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Table 15-1 List of major taste and odor causing algal volatile organic compounds (VOCs), with odor descriptors (where known), known producers listed with their taxonomic groups: CY = Cyanobacteria; GR = Chlorophyta (green algae), CHR = Chrysophyta (Diatoms [Dia], Chrysophyceae [Chr] and Synurophyceae [Syn]), DN = Dinophyta; CR = Cryptophyta, RD = Rhodophyta (continued)

			Taxicological Group								
					CHR						
VOC	Reference	Descriptor	CY	GR	Dia	Chr	Syn	DN	CR	RD	Taxa
Chlorophene	7	Phenolic	x				x				*Anabaena laxa; Oscillatoria* sp.; *O. tenuis; Phormidium* sp.; *Synura petersenii*
Cieneol (1,8)	5, 22	Camphor, spicy, cool		x							*Scendesmus subspicatus; Chlorella sorokiana*
Dihydroactinidiolide	25, 30	Pungent violet; tealike	x	x							*Synechococcus; Scenedesmus* sp.; *C. vulgaris, Chara globularis*
Geosmin	7, 13, 20, 25, 26	Earthy/musty	x								See Table 15-3
Geraniol	6, 30	Sweet, floral, fruity	x	x							*Synechococcus PCC6911; C. vulgaris*
trans-Geranyl acetone	5, 9	Flowery		x						x	*Scenedesmus subspicatus; Cyanidium caldarium*
Tetrahydrogeranylacetone	25		x								*Synechococcus*
Germacrene-D	20, 28	Grassy	x	x							*Anabaena flos-aquae; A. macrospora; Oscillatoria* sp.; *Oscillatoria splendida; Microcystis; Scenedesmus*
Hexahydrofarnesyl acetone				x							*Chara globularis*
α-Ionone	5, 30	Violets		x							*Scenedesmus subspicatus; Chlorella vulgaris, Scenedesmus* sp.
β-Ionone	5, 8, 9, 14, 30	Violets	x	x			x		x	x	*Scenedesmus subspicatus; Anabaena* sp.; *A. cylindrica; Aphanizomenon gracile; Synechococcus 6911; S. uvella; C. caldarium, Chlorella vulgaris, Scenedesmus* sp.
Epoxy-β-ionone	30	Violets		x							*Chlorella vulgaris, Scenedesmus* sp.
Limonene	5, 22	Citrus		x	x						*S. delicatissima; Chlorella sorokiniana; C. homosphaera*

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Table 15-1 List of major taste and odor causing algal volatile organic compounds (VOCs), with odor descriptors (where known), known producers listed with their taxonomic groups: CY = Cyanobacteria; GR = Chlorophyta (green algae), CHR = Chrysophyta (Diatoms [Dia], Chrysophyceae [Chr] and Synurophyceae [Syn]), DN = Dinophyta; CR = Cryptophyta, RD = Rhodophyta (continued)

			Taxicological Group								
					CHR						
VOC	Reference	Descriptor	CY	GR	Dia	Chr	Syn	DN	CR	RD	Taxa
Linalool	5, 30	Grassy, floral		x							*S. subspicatus, Chlorella vulgaris*
Menthone	6	Mint									Diatom biofilms
Methylgerianate	9								x	x	*Cyanidium caldarium*
Myrcene	22	Pleasant odor		x							*Chlorella homosphaera*
Nerol (2-*cis*-3,7-dimethyl-2,6-octadien-1-ol)	25	Sweet, roselike; bitter flavor	x								*Synechococcus PCC6911*
Phytol (tetramethyl 3,7,11,15-hexadec-2-en-1-ol)	7	Delicate floral balsam	x	x							*Anabaena laxa; C. peterfii; M. aeruginosa; Oscillatoria* sp.; *C. vulgaris*
Squalene	7	Faint, agreeable	x				x				*A. laxa; M. aeruginosa; Oscillatoria* sp.; *O. tenuis; Phormidium* sp.; *D. cylindricum; S. petersenii*
Styrene	8	Sweet, balsamic			[x]		x				*Synura uvella*
Oxylipins and Other Hydrocarbons											
3-Methyl butanal	5, 8b	Rancid/putrid		x		x					*Ochromonas danica; M. aeruginosa*
2-Butanone	8b, 36	Ethereal	x			x					*Ochromonas danica, Nostoc PCC6314, Nostoc PCC6310*
cis-2-Octene	8b					x					*Ochromonas danica*
2,4-Decadienal (*trans* and *cis* isomers)	6, 8a, 13, 2, 9	Fishy, rancid			[x]	x	x	x	x		*P. malhamensis; Dinobryon* spp.; *U. americana; S. uvella; S. petersenii; Cryptomonas rostratiformis; Peridinium willei; Mallomonas papillosa*
2,4,7-Decatrienal (*trans* and *cis* isomers)	30	Fishy, swampy			x	x	x				*Fragilaria* sp.; *Dinobryon* spp.; *U. americana; S. uvella; S. petersenii; M. papillosa*

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Table 15-1 List of major taste and odor causing algal volatile organic compounds (VOCs), with odor descriptors (where known), known producers listed with their taxonomic groups: CY = Cyanobacteria; GR = Chlorophyta (green algae), CHR = Chrysophyta (Diatoms [Dia], Chrysophyceae [Chr] and Synurophyceae [Syn]), DN = Dinophyta; CR = Cryptophyta, RD = Rhodophyta (continued)

			Taxicological Group								
					CHR						
VOC	Reference	Descriptor	CY	GR	Dia	Chr	Syn	DN	CR	RD	Taxa
Dictyopterene C' (6-butyl-cyclohepta-1,4-diene)	33	Seaweed			x						*A. veneta; G. parvulum*
Dictyopterene A [*trans* 1 -(*trans* 1-hexenyl)-2-vinylcyclopropane]	33	Seaweed			x						*A. veneta; G. parvulum*
Ectocarpene (6-(Z1-butenyl)-1,4-cycloheptadiene)	33	Tomato-leaf-like			x						*Amphora veneta; Gomphonema parvulum*
Ethanol	2						x				*S. petersenii*
Ethyl acetate	2	Pineapple, ethereal					x				*S. petersenii*
Ethyl propionate	4	Sweet, fruity, fragrant, ethereal	x								*Anabaena laxa; Phormidium*
n-Heptadecane	7, 8, 20, 32	Nonodorous	x	x		x					*Oscillatoria* sp.; *O. tenuis; O. redekei Anabaena flos-aquae; A. laxa; M. aeruginosa; Phormidium* sp.; *S. petersenii; Chara globularis*
1-Heptadecene	30			x							*Scenedesmus* sp.; *C. vulgaris*
Heptadec-*cis* 5-ene	13		x								*Aphanizomenon gracile; Planktothrix redekeii*
2,4-Heptadienal (*trans* and *cis* isomers)	11, 29, 36	Fishy; cod liver oil	x		x	x	x				*Uroglena americana; Anabaena oscillarioides; Fragilaria* sp.; *Melosira varians; M. papillosa*
n-Heptanal	8b, 34	Strong, fatty, pungent, fishy	x	x			x		x		*Synura uvella; Cryptomonas ovata var palustris*
n-Heptane	8b	Sweet-ethereal			[x]						*Synura uvella*

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Table 15-1 List of major taste and odor causing algal volatile organic compounds (VOCs), with odor descriptors (where known), known producers listed with their taxonomic groups: CY = Cyanobacteria; GR = Chlorophyta (green algae), CHR = Chrysophyta (Diatoms [Dia], Chrysophyceae [Chr] and Synurophyceae [Syn]), DN = Dinophyta; CR = Cryptophyta, RD = Rhodophyta (continued)

			Taxicological Group								
					CHR						
VOC	Reference	Descriptor	CY	GR	Dia	Chr	Syn	DN	CR	RD	Taxa
1-Heptanol	6	Fresh, light green, nutty	x								*Synechococcus* 6911
2-Heptanol	10	Fruity-green, herbaceous				x					*Ochromonas danica*
2-Heptanone	10	Banana, slightly spicy				x					*Ochromonas danica*
n-Hexanal	5, 8a, 13, 15, 36	Grassy, fatty	x	x	x		x				*S. subspicatus; Stephanodiscus hantzschii; S. delicatissima; A. granulata; S. uvella; Anabaena oscillarioides*
1-Hexadecane	30			x							*Scenedesmus* sp.*; C. vulgaris*
n-Hexanol	5, 36	Grassy	x	x							*S. subspicatus; Anabaena oscillarioides*
1-Hexanol	5, 6, 36	Grass cuttings	x	x							*Synechococcus; S.* 6911; *S. subspicatus, Nostoc* PCC6314
cis-3-Hexen-1-ol	5	Grass cuttings		x							*S. subspicatus*
Isoamyl acetate (3-methyl-1-butyl acetate)	2	Banana					x				*S. petersenii*
Isobutyl acetate (2-methylpropyl ethanoate)	8a				[x]		x				*S. uvella*
Isobutyl alcohol	2						x				*S. petersenii*
Isopropyl acetate (isopropyl ethanoate)	10	Fruity				x					*O. danica*
Isopropyl alcohol	2, 6, 10					x	x				*P. malhamensis; S. petersenii; O. danica*
Methyl acetate (methyl ethanoate)	2	Glue, nail polish remover					x				*S. petersenii*

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Table 15-1 List of major taste and odor causing algal volatile organic compounds (VOCs), with odor descriptors (where known), known producers listed with their taxonomic groups: CY = Cyanobacteria; GR = Chlorophyta (green algae), CHR = Chrysophyta (Diatoms [Dia], Chrysophyceae [Chr] and Synurophyceae [Syn]), DN = Dinophyta; CR = Cryptophyta, RD = Rhodophyta (continued)

			Taxicological Group								
					CHR						
VOC	Reference	Descriptor	CY	GR	Dia	Chr	Syn	DN	CR	RD	Taxa
Methyl butanol	6					x					*P. malhamensis*
Methyl formate (methyl methanoate)	8b	Ethereal				x					*Tetraselmis* bloom
Methyl isobutyrate	2					x					*S. petersenii*
Methyl n-butyrate	8a,b	Pineapple, apple	x		x		x				*S. uvella; A. formosa*
Methyl octadecanoate	35			x							*Scenedesmus* sp.; *C. vulgaris*
Methyl octadec-9-enoate	35			x							*Scenedesmus* sp.; *C. vulgaris*
Methyl propionate	2, 4	Pungent	x			x	x				*Synechococcus* 6911; *O. danica; S. uvella*
3-Methyl-1-butanol (isoamyl alcohol)	10, 20	Smoky, moldy; fusel oil	x			x					*M. aeruginosa, Ochromonas danica*
3-Methylbutan-2-one	10					x					*Ochromonas danica*
3-Methylbut-2-enal	10	Rancid; putrid				x					*Ochromonas danica*
2-Methylbut-2-en-1-ol	10						x				*Ochromonas danica*
Methyl octadec-9,12-dienoate	35			x							*Scenedesmus* sp.; *C. vulgaris*
2-Methylpent-2-enal	8b	Green apples		x							*Tetraselmis* bloom
2-Methylpropan-1-ol	10						x				*Ochromonas danica*
n-Octadecane	7		x			x					*M. aeruginosa; D. cylindricum; Oscillatoria* sp.; *Phormidium* sp.
n-Nonadecane	8a, 14				x		x				*S. uvella; A. formosa*
2,6 -Nonadienal (*trans* and *cis* isomers)	5, 7, 20, 24	Grassy; cucumber		x			x				*S. petersenii; Scenedesmus subspicatus*
Octadecane	30			x							*Scenedesmus* sp.; *C. vulgaris*
Octadecene	7, 32		x	x							*Phormidium* sp.; *Chara globularis*

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			Taxicological Group								
					CHR						
VOC	Reference	Descriptor	CY	GR	Dia	Chr	Syn	DN	CR	RD	Taxa
2,4 -Octadienal (*trans* and *cis* isomers)					x						*A. granulata; Fragilaria*
1,3-Octadiene	14, 36	Earthy, mushroom; humus	x		x						*A. formosa; Anabaena oscillarioides*
Octa-1,5-dien-3-ol	36		x								*Anabaena oscillarioides*
Octan-1-ol	6, 8b, 10, 36	Sweet, fresh, orange-rose	x		[x]	x	x				*Synechococcus* 6911; *Ochromonas danica; S. uvella; M. aeruginosa; D. cylindricum; Dinobryon* sp.; *Oscillatoria* sp.; *Phormidium* sp., *Anabaena oscillarioides*
Octan-3-ol	8b	Nutty, warm, herbaceous			[x]		x				*S. uvella*
Octane	10					x					*O. danica*
Octan-3-one	8a, 36	Strong, fruity, lavender	x				x				*P. malhamensis, S. petersenii, Anabaena oscillarioides*
Octadiene	8b	Geranium			x						*A. formosa*
1,3,5-Octatriene	8b, 36	Geranium	x		x						*A. formosa; Anabaena oscillarioides*
2,4,6-Octatriene	36		x		x						*Anabaena oscillarioides*
2-Octenal (*trans* and *cis* isomers)	6, 8a, 36	Green-leafy, orange, honey	x			x	x				*P. malhamensis, S. petersenii, Anabaena oscillarioides*
trans-2-Octene	8b,10				[x]	x	x				*Synura uvella; Ochromonas danica*
Oct-1-ene	8b				[x]	x					*Synura uvella; Synechococcus* 6911; *Anabaena oscillarioides*
Octan-3-ol	36		x								*Anabaena oscillarioides*
Oct-2-en-1-ol	10, 36		x			x					*Ochromonas danica, Anabaena oscillarioides*

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			Taxicological Group								
					CHR						
VOC	Reference	Descriptor	CY	GR	Dia	Chr	Syn	DN	CR	RD	Taxa
Oct-1-en-3-ol	10, 36	Sweet, earthy, herbaceous, mushroom				x					*Ochromonas danica, Anabaena oscillarioides*
Oct-1-en-3-one	8b, 36	Mushroom	x		[x]		[x]				*Synura uvella, Anabaena oscillarioides*
n-Pentanal	5, 34	Fishy			x				x	x	*Aulacoseira granulata, Cryptomonas ovata var. palustris*
Methyl pentanoate	2	Green-fruity, apple-pineapple					x				*S. petersenii*
1-Pentanol	14, 36	Harsh, faint fusel oil	x								*A. cylindrica, Nostoc* PCC6314
2-Pentenal (*trans* and *cis* isomers)	14		x				x				*S. uvella; Anabaena cylindrica;*
Pent-1-en-3-ol	14		x								*Anabaena cylindrica*
Pent-1-en-3-one	5, 8, 14	Pungent		x	x		x				*Aulacoseira granulata; S. subspicatus; S. uvella*
2-Pentylfuran	8a	Buttery, green bean					x				*S. petersenii*
2-Propanone (acetone)	34, 36	Acetone	x						x		*Cryptomonas ovata var palustris, Nostoc* PCC6314, *Nostoc* PCC6310
Propenal	8a	Acrid, burning fat			[x]		x				*S. uvella*
n-Propyl acetate (propyl ethanoate)	8a	Pears			[x]		x				*S. uvella*
2-Tridecanone	30			x							*C. vulgaris*

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			Taxicological Group								
					CHR						
VOC	Reference	Descriptor	CY	GR	Dia	Chr	Syn	DN	CR	RD	Taxa
6,10,14-Trimethylpenta-decan-2-one	30			x							*C. vulgaris*
Undecan-2-one	30	Sweet, slightly peachy				x					*Scenedesmus* sp.; *C. vulgaris*

NOTE: Brackets [x] denote trace amounts.

References: 1 = Herrmann and Jüttner 1977; 2 = Collins and Kalnins 1965; 3 = Burlingame et al. 1992; 4 = Jüttner et al. 1982; 5 = Cotsaris et al. 1995; 6 = Jüttner and Hahne 1981; 7 = Rashash et al. 1995; 8a = Jüttner 1981a; 8b = Jüttner 1981b; 9 = Jüttner 1979; 10 = Jüttner et al. 1982; 11 = Yano et al. 1988; 12 = Krasner et al. 1985; 13 = Jüttner et al. 1986; 14 = Jüttner 1983; 15 = Jüttner 1984; 16 = Anthoni et al. 1980; 17 = Wium-Andersen et al. 1982; 18 = Bechard and Rayburn 1979; 19 = Tsuchiya and Matsumoto 1988; 20 = Hayes and Burch 1989; 21 = Jüttner and Muller 1979; 22 = Liersch 1976; 23 = Krasner et al. 1985; 24 = Wee et al. 1994; 25 = Henatsch and Jüttner 1983; 26 = Wnorowski 1992; 27 = Means and McGuire 1986; 28 = Jones and Korth 1995; 29 = Watson et al. 1999a; 30 = Rzama et al. 1995; 31 = Khiari et al 1995; 32 = Berger and Schagerl 2004; 33 = Jüttner and Wurster 1984; 34 = Collins and Kalnins 1965; 35 = Rzama et al. 1995; 36 = Mohren and Jüttner 1983; 37 = Tsuchiya et al. 1979, referenced in Höckelmann et al. 2004; 38 = Brownlee et al. 1984.

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Modes of Odor Production

Several general points should be made, before examining the chemicals and biota in more detail. Algae cause or contribute to T&O

- *Directly*, via

1. VOCs produced *during growth* and excreted to surrounding water, and/or stored and released later during internal changes in cell metabolism or damage/death via external agents (e.g., invertebrate predators, parasites, temperature, pH, photolytic or osmotic stress [UV, dehydration], treatment application of algicides, chlorine, ozone, etc);
2. VOCs synthesized *only at cell death or damage* (e.g., via the above external agents) generated from catalytic enzymes activated by this lysis; and

- *Indirectly*, with the *postmortem* breakdown of their cells by microorganisms, which can produce intense, rank, putrid, or even fecal-smelling T&O, for example, during the degradation of beached and disintegrating algal mats (Brownlee et al. 1984). This manner of odor generation can involve both algal species that do, and do not, directly produce VOCs themselves—and, of course, other rotting organic material from aquatic and terrestrial sources.

While all or some of these various modes of VOC production can occur over the lifespan of a cell, they differ in (1) their major biochemical pathways, (2) triggers and controls, and (3) timing—all of which have important implications for correct sampling, analytical protocol, and data interpretation.

Variation Among Species/Strains

VOC chemistry and per capita production can vary significantly (1) among strains of the same species, and (2) among (even closely related) species, all of which can be isolated from different or the same waters and may or may not co-occur. Conversely, some of these species may produce the same VOC chemistry.

Common VOC Classes and Their Biological Producers

Since many VOCs are produced by several algal species and taxonomic groups (Table 15-1), they cannot be conveniently categorized. Nevertheless, there are some general patterns in VOC origins, synthesis, and chemistry that can produce different odor types with characteristic seasonal patterns.

Cyanobacteria ("blue-green algae") are widely known for earthy/muddy/grassy/sulfurous odors caused by highly potent terpenoids, sulfides, and hydrocarbons, mostly synthesized during growth.

In contrast, many Chrysophyta* (i.e., "golden-brown" algae, including Bacillariophyceae [diatoms], Chrysophyceae, and Synurophyceae) are known for odorous lipid derivatives, produced during cell breakdown, and often causing "fishy/rancid" or "cucumber" smelling events. Some haptophytes†—notably *Chrysochromulina*—are also known to produce fishy T&O, but their VOCs are yet to be chemically characterized. Two important groups of unicellular flagellates, Dinophyta and Cryptophyta, also produce some of these lipid derivatives and other carbonyl compounds.

Green algae (Chlorophyta) are most noted for grassy-smelling fatty acid derivatives (notably leaf alcohols) and terpenoids. Red algae or Rhodophyta are less common

* The current classification of Chrysophyta includes these and other related classes, with prymnesiophytes now in the Haptophyta (e.g., Andersen 1987).

† Also called Prymnesiophyta. Previously included under the Chrysophyta.

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to inland waters, although a few are highly successful, and the single member of this group examined to date has been found to produce floral-smelling pigment derivatives. Little or no information exists on odors produced by the Euglenophyta, which are typically found in organically enriched waters such as waste treatment ponds. Each of these groups also produces minor amounts of other VOCs that may contribute to the quality of the odor.

Establishment of Biological Producers

Many of the VOCs and species listed in Table 15-1 were identified using a variety of sampling and analytical techniques, and/or culture conditions. In recognition of the need to apply standard protocols, a series of criteria were proposed to confirm species and compounds as primary causes of an odor event (Persson 1983):

1. Co-occurrence of species, odor, and VOCs in the field;
2. VOC production by cultures of the same species isolated from the same field samples; and
3. Analytically determined chemical and sensory properties of the VOCs.

However, resources and expertise are often not available for this time-consuming and costly procedure (see Izaguirre 2007). Moreover, several factors may produce ambiguous or misleading test results:

- Strains obtained from a field population containing producers and nonproducers of the same (or morphologically similar) species;
- Changes or loss of VOC production capacity induced by nonideal culture conditions;
- Chemistry and dynamics of VOC production, cell storage, and release;
- Spatial/temporal decoupling of VOCs and producers in the field by large-scale physical processes.

With sufficient insight into the nature of the source water, the taxa, and the compounds, these issues can be minimized.

It is helpful to first consider the basic chemistry, biosynthetic routes, and properties of algal VOCs, focusing on those compounds that have widespread and significant impacts on surface water T&O. As will be more specifically discussed below, VOCs may be released and/or stored by cells, and vary among and within species in both their chemistry and production rate. Different species can generate a diversity of VOCs with similar or distinct biochemical pathways, chemistry, potency, stability, and production rates, all of which may differ with growth stage and/or with environmental conditions. This complexity translates to radically different degrees of VOC persistence in surface waters, and response to treatment. The following section will first briefly discuss general VOC chemical and physical properties and why these are important, and then focus on major chemical groups that have the most widespread effects on aquatic odor—terpenes, fatty acids, biogenic sulfides, and (possibly) amines—and examine biosynthetic mode, chemistry, odor, algal producers, and other important properties that influence their occurrence and dynamics in source waters.

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CHEMISTRY

VOC Properties and General Sampling/ Analytical Considerations

The odor and chemistry of an off-flavor episode provide important clues to the biological (or other) sources, and the dynamics of these events (e.g., Preti et al. 1993). It is important to consider the properties of suspected or "candidate" VOCs when attempting to identify the chemical and biological sources of odor, for several reasons.

1. *VOC properties are highly relevant to the choice of analytical protocols and to data interpretation.*
2. *The nature of an odor provides a valuable clue to its chemical and biological origins*, especially if combined with information about the source water and dynamics of the T&O event. Sensory analysis (e.g., flavor profile analysis, FPA) is a practical, cost-effective way of screening samples and detecting and monitoring T&O events, and is successfully applied throughout the water industry (Krasner et al. 1985, Bartels et al. 1986, Dietrich et al. 2004). It provides a qualitative assessment of odor characteristics and intensity, which (for the more informed sensory analysts) can also point to culprit VOCs and biota. However, these techniques clearly cannot identify or quantify the compounds and can be problematic where a mix of VOCs is present and/or the concentrations of the more potent compounds vary over time (see item 11 below and Algal Odor Compounds section).
3. *Analytical and sensory analyses of mixtures are not always well correlated.* In part, this is because perceived odor may not necessarily be caused by the dominant VOC and may be modified by synergistic combinations of different compounds. Highly volatile compounds present at levels that are below analytical detection may mask or change the odor of other VOCs in a sample, and in fact chlorine can have a similar effect in treated water (Dietrich et al. 1992, Rashash et al. 1996, Oestman et al. 2004).
4. *Efficient extraction and concentration techniques are required for identification and quantification,*[*] since most VOCs are present at ultra-trace concentrations. Inevitably, these techniques give differential recovery of compounds with different chemical and physical properties that often co-occur in samples.
5. *Confidence limits around measurements are rarely reported but can range upward of ±10%–20%*, and interlab comparisons can show a wide discrepancy in reported levels (Brownlee et al. 2007). This is an important consideration when attempting to interpret data from different labs or seasonal and spatial changes.
6. *Samples from planktonic blooms, runoff events, benthic mats, biofilms, and cultures often have a significant particulate fraction that can be especially problematic.* Incomplete recovery can significantly underestimate the actual VOC content, bias the results toward more readily extracted compounds, and generate an apparent mismatch between the biomass of the producers and measured VOC levels. With these organicially rich samples, analyte recovery is markedly reduced by two factors: adsorption of dissolved compounds onto

[*] For example, liquid-liquid solvent extraction, simultaneous distillation extraction, closed or open loop stripping, liquid- and solid-phase microextraction, or stir bar adsorption (Cotsaris et al. 1995, Khiari et al. 1995; Bao et al. 1997; Jeannot and Cantwell 1996; Watson et al. 1999a, 1999b; Benanou et al. 2004; Brownlee et al. 2007.

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sample matrices and incomplete recovery of the particle-bound VOCs. The latter is often overlooked, but in fact many current analytical techniques do not fully extract the particulate phase, which requires the disruption of algal cells (e.g., via salting, ultrasonification, or freeze–thaw), sometimes in conjunction with polar solvents. This is a particular concern for VOCs that are tightly bound to cell material, such as geosmin and 2-methylisoborneol (MIB) (Jüttner and Watson 2007; see item 7).

7. *Analytical protocols can modify the chemical profile of a sample* by introducing background contaminants, generating chemical artifacts, and/or altering the algal VOC chemistry of a sample. For example, the terpenoid geosmin can be converted to nonodorous argosmin by dehydration (Jüttner 1995). With lipid-rich taxa, analytical procedures can erroneously measure "free" (i.e., not cell-bound) polyunsaturated fatty acids (PUFAs; Jüttner 2001). In addition, it has been shown that cell damage triggers the synthesis of odorous PUFA and pigment derivatives by activating catalytic enzymes (see Fatty Acids and Oxylipins sections). Since most analytical procedures induce some cell lysis (but see item 6), measurements of these particular compounds, in essence, represent the *potential* odor of a sample—which may not be manifested under normal conditions.

8. *VOC properties influence their persistence and fate* in a water body and stored samples.

9. *VOC properties largely determine potency or odor threshold concentration (OTC).* OTCs are not simply a function of molecular weight but vary with chemical structure (see Eriksson et al. 1976, Weyerstahl 1994, Young et al. 1996). OTC estimates for algal odor compounds a range over six orders of magnitude* (Table 15-2; Figure 15-2). Not surprisingly, VOCs with the lowest OTCs are most pervasive and most frequently reported (e.g., geosmin, MIB). These differences in potency mean that

   a. Odor is not necessarily caused by the most abundant VOC and may be modified by masking effects or synergistic combinations in VOC blends; and

   b. Species with a low per capita production of a highly potent compound can have a significant effect on odor levels, and in a mixed algal community, the primary odor source is not necessarily the dominant species (e.g., Watson and Satchwill 2003). It should be also noted that *measures of algal standing stock as cell counts are misleading* unless weighted for the substantial differences in size among species (typically from <1 μm to ~250–500 μm in maximum dimension, but up to ~10 cm for *Chara* and other odorous green macroalgae). More representative comparisons are made using biovolume as a measure of algal abundance (see Chapter 2).

10. *For some compounds, the character of the perceived odor changes with increasing concentrations.* For example, *trans*, *cis*, 2,6-nonadienal has a "sweet, green cucumber" odor at 6 ng/L, but "waxy, oily, rancid" at 60 ng/L (Burlingame et al. 1992). Changes in aquatic concentrations (e.g., during the wax and wane of algal populations) might lead to the conclusion that two or more VOCs are involved.

* Although it is noted that these values were determined by different sniffing panels under different conditions.

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Table 15-2 Survey of odor threshold concentrations (OTC; mg/L) and odor descriptives reported for algal metabolites. OTCs for compounds within major chemical categories are shown in ascending value. Arrows indicate changes in odor descriptives with increasing concentration. (Source: Krasner et al. 1985, Mallevialle and Suffet 1987, Rashash et al. 1996, Young et al. 1996, Satchwill et al. 2007.)

Compound	OTC	Odor
Aromatics		
3-Methoxy-2-isopropyl pyrazine	0.0002	Earthy/potato bin
2-Isobutyl-3-methoxy pyrazine	0.001	Earthy/potato bin
2,6-Dimethyl pyrazine	6	Cocoa, roasted nuts, coffee
Benzyl acetate	30	Artificial banana
2-Phenylethanol	45	Floral rose
Sulfides		
Dimethyl trisulfide	0.01	Septic, garlic, putrid, swampy
Dimethyl disulfide	<4.0	Septic, garlic, putrid
Terpenoids		
2-Methylisoborneol	0.002–0.02	Earthy/musty
Geosmin	0.006–0.01	Earthy/musty
Epoxy-α-ionone	0.007	Floral
α-Ionone	0.007	Violets
β-Ionone	0.007	Violets
3-Methylbut-2-enal	0.15	Rancid/putrid
3-Methyl butanal	0.15	Rancid/putrid
Cieneole (1,8)	12	Camphor, spicy, cool
Limonene	4	Citrus
Linalool	6	Grassy, floral
β-Cyclocitral	19.3	Hay/grass → tobacco, smoky/moldy
6-Methyl-5-hepten-2-one	50	Fruity; esterlike
Geranyl acetone	60	Floral
Terpenoids (continued)		
Styrene	65	Sweet, balsamic
2,2,6-Trimethyl cyclohexanone	310	Tobacco/musty
2,2,6-Trimethyl cyclohex-2-en-1-one	420	Tobacco, potato cellar
Camphor	1290	Aromatic, musty, woody
1,3,3-Trimethyl cyclohexene	7100	Tobacco
Fatty Acids, Oxylipins		
trans-2, *cis*-6-Nonadienal	0.08	Grassy; cucumber → oily/rancid
trans-2-Nonenal	0.8	Cucumber
1-Penten-3-one	1.25	Pungent; rancid; fishy
trans, *cis*, *cis*-2,4,7-Decatrienal	1.5	Fishy, oily
n-Heptanal	3	Fishy, oily
n-Hexanal	4.5	Grassy, fatty
trans, *cis*-2,4 Heptadienal	5	Fishy, oily
Linolenic acid	5	Watermelon
trans-2-Hexenal	17	Green leaf
3-Methyl-butyrate	20	Rotten, rancid
n-Pentanal	60	Fishy
cis-3-Hexen-1-ol	70	Grassy
n-Hexanol	200	Green leaf
2-Methylpent-2-enal	290	Rum, marzipan
1,3-Octadiene	5600	Earthy, mushrooms

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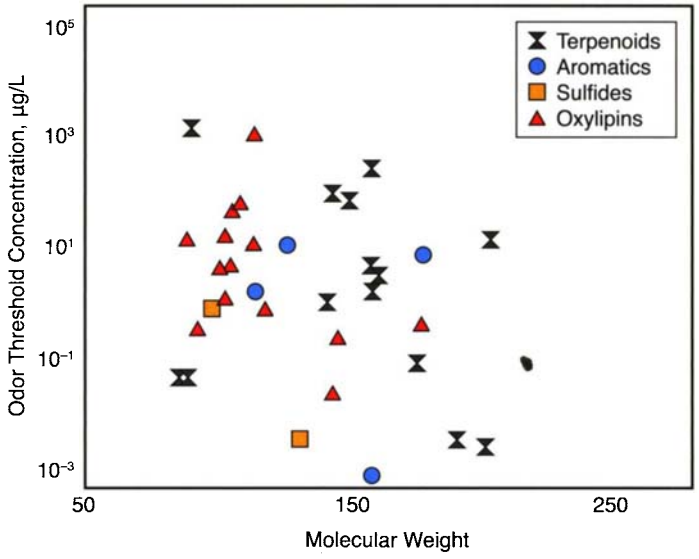

Figure 15-2 Odor threshold concentrations of major algal VOCs as a function of molecular weight

11. *Many VOCs have more than one structural isomer with different organoleptic properties*, while OTCs, odor descriptors, and chemical analyses are typically based on commercially available analytical standards, which are usually in one molecular configuration or as racemic mixes (see Oxylipins: Production and Properties section). Interconversion among isomers in samples (e.g., via light) can change sample odors and gas chromatography profiles (Jüttner 1983, Yano et al. 1988; see Muddy/Musty Odors: Geosmin and MIB section).

ALGAL ODOR COMPOUNDS: PATHWAYS, PROPERTIES, AND PRODUCERS

It is beyond the scope of this chapter to provide comprehensive descriptions of the biosynthesis, chemistry, and structures of algal VOCs and the physiological ecology of individual algal producers. These are covered in more detail by several recent reviews and the references therein. The aim of this section is to provide a synopsis of the major compounds and species involved in most T&O events, with references to more specific papers for the interested reader. A number of reviews of algal T&O summarize current knowledge in these subjects (e.g., Jüttner 1995; Persson 1996; Watson 2003, 2004; Watson et al. 2001a; Jüttner and Watson 2007; Izaguirre 2007).

Terpenes

Terpenes (multiples of isoprene or 2-methyl, 1,3-butadiene; Figure 15-3) and derivatives (terpenoids) are a large group of secondary metabolites synthesized by a diversity of organisms. Terpenes have a wide variety of odors (e.g., in the essential oils of many higher plants) and play important roles in chemical signaling in terrestrial and aquatic food webs.

Many terpenes are produced and stored or excreted during active growth, and in some cases, the bulk release or synthesis may occur at the stationary phase or senescence (below). In fact, the partitioning of extra- vs. intracellular production varies considerably between different species and under different environments. Two freshwater

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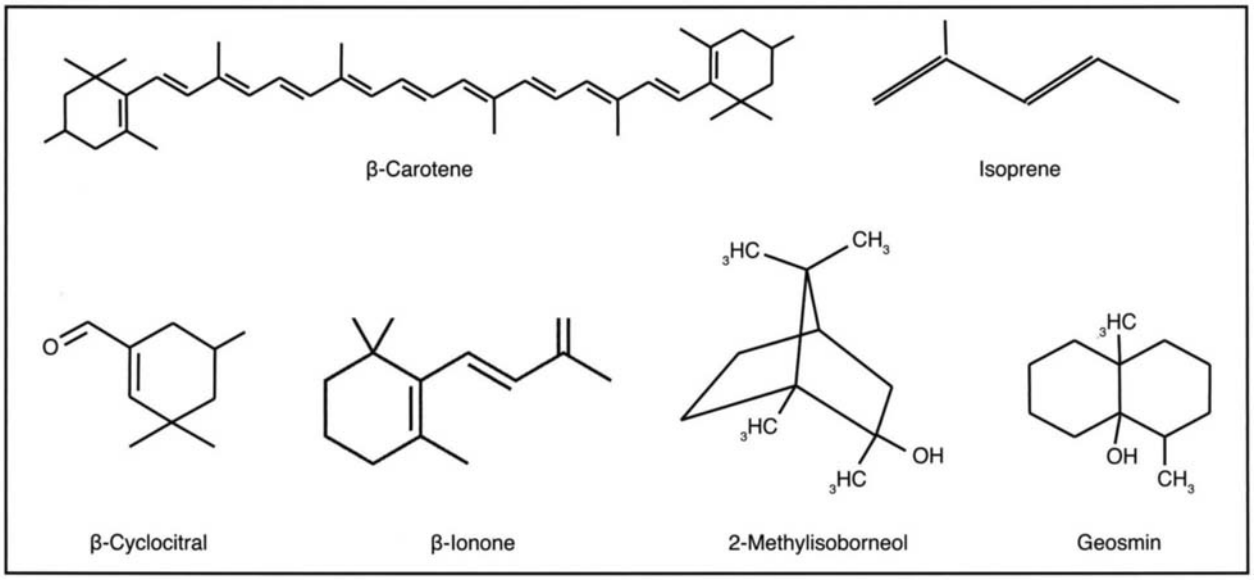

Figure 15-3 Chemical structures of isoprene and major odorous terpenes and terpenoids produced by algae and cyanobacteria

algal groups, cyanobacteria and Chlorophyta, produce significant levels of terpenes (Jüttner 1995), with some production by a few other algal groups (chrysophytes, synurophytes, and diatoms; Cotsaris et al. 1995; Rashash et al. 1996; Table 15-1).

Muddy/musty odors: geosmin and MIB. Geosmin (*trans*-1, 10-dimethyl-*trans*-9-decalol) and MIB (2-methylisoborneol) are two highly volatile cyclic sesquiterpenoids (Figure 15-3) that account for the majority of reported T&O events (e.g., Persson 1996, Wnorowski 1992, Jones and Korth 1995, Jüttner 1995, Jüttner and Watson 2007, and many others). Considerable research has focussed on these notorious compounds and our knowledge of their chemistry, molecular properties, biotic sources, and behavior in raw and treated water is extensive (see, e.g., Izaguirre 2007, Jüttner and Watson 2007). Nevertheless, there are some important outstanding questions, and these compounds are still implicated in many unsolved T&O outbreaks. Because of their prominence among odor compounds, geosmin and MIB will be discussed in some detail in this section.

Geosmin and MIB have very low OTCs (~4–10 ng/L; Table 15-2), and impart earthy/musty T&O to water. These compounds are relatively stable and may persist in the water column for some time after release, allowing VOC movement away from the production site. They are, however, biodegraded by some heterotrophic microbes, both *in situ* and on biologically active filter beds. Geosmin and MIB resist conventional water treatment (sand filtration and chlorination) and require advanced processes such as activated carbon, ozonation, membrane filtration, and other treatment (e.g., Yagi et al. 1988, Tanaka et al. 1996, Saito et al. 1999, Cook and Newcombe 2004, Bruchet and Laîné 2005, Park et al. 2006).

Both terpenoids exist as (–) and (+) chiral forms that differ in potency: the naturally occurring (–) enantiomers* are about ten times more potent than the commercially synthesized (+) forms, which may account for differences in consumer response thresholds and reported OTCs (Tyler et al. 1978, Polak and Provasi 1992). As noted, recovery of these compounds by many extraction methods is often incomplete. These highly volatile compounds may be lost by volatilization or microbial activity, or converted to odorless derivatives during storage, handling, or analyses (e.g., Sumitomo

* Nonsuperimposable mirror images.

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1992, Jüttner 1995, Saito et al. 1999). To identify and monitor these compounds at their threshold levels, consistent analytical techniques are needed that are ~10× more sensitive than any enzyme-linked immunosorbent assay (ELISA) developed to date (see Chung et al. 1991).

Cyanobacteria are the only known producers of geosmin and MIB among "algae."* These compounds are not produced by eukaryotic or "true" algae, although they are synthesized by some bryophytes and higher plants (Watson 2003). It should be noted, however, that *not all cyanobacteria produce these compounds or other strong odors*; in fact, fewer than 50 of the >2,000 species classified to date have been directly confirmed as geosmin and/or MIB producers.

Taxonomy, growth phase, and environment influence geosmin and MIB production, but the relative importance of each factor is not resolved. Early studies concluded that these compounds are by-products of carotenoid synthesis, since production is modified by factors such as light and nutrients, which change cell pigment content (e.g., Naes et al. 1988, Blevins et al. 1995, Rashash et al. 1995). Recent work now indicates that geosmin and MIB are coupled with more fundamental cell energetics (Utkilen and Frøshaug 1992, Zimba et al. 1999), which may or may not be linked with light. In fact, population growth cycles, taxonomic affiliation, and species succession may have more influence on the per capita geosmin or MIB output by an algal population than factors such as light and nutrient levels (Naes et al. 1988). There is considerable variance among taxa in their production capacity and internal/external partitioning, with nonproducers and producers even among strains of the same species (e.g., Oikawa and Ishibashi 2004, Izaguirre 2007). Culture work has shown, for example, a decrease in cell-bound geosmin and MIB content with growth rate and population age—but only for some species. To some extent, this variance may result from applying suboptimal culture conditions to strains with different optimal environments. For example, under the same conditions, the cellular geosmin yield of *Phormidium* sp. was ca. four times that of *Anabaena laxa* and *Oscillatoria tenuis*† (Rashash et al. 1995). Geosmin is not produced under culture by all isolates of *Synechococcus* clones (Izaguirre et al. 1982), while only brackish water clones of *Oscillatoria aghardhii*‡ produced geosmin and MIB among fresh and brackish water isolates (Persson 1982).

Geosmin and MIB are synthesized during active growth. In most cases, the bulk of this production is retained in the cells, tightly associated with membrane proteins. MIB is less closely bound and more readily excreted than geosmin (Wu and Jüttner 1988, Jüttner and Watson 2007). Most healthy cells release a relatively small fraction of these compounds into the environment. Cell-bound and dissolved fractions respond very differently to treatment, and their relative proportions have major implications for data interpretation, yet these fractions are rarely resolved or considered by researchers and managers. Several factors can alter concentrations of dissolved and particulate geosmin and/or MIB over the course of an odor event, notably:

- Succession among species with different production and allocation to internal and external fractions;
- Population growth stage;
- Environmental conditions;

* For brevity, eukaryotic algae and cyanobacteria will be collectively referred to as "algae" unless indicated; note that cyanobacteria belong to the bacteria Monera and differ in reproduction, cell structure, and much of their biochemistry from true algae (Chlorophyta, Chrysophyta, Cryptophyta, Dinophyta, Euglenophyta, Haptophyta, etc.).

† Syn. *Phormidium tenue*.

‡ Syn. *Planktothrix aghardhii*.

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- Cell death or damage by abrupt environmental changes (e.g., temperature, irradiance, salinity, dessication), or grazers and pathogens. Zooplankton, for example, can liberate significant amounts of cell-bound geosmin in surface layers, while microbial activity and benthic grazers can generate elevated levels in bottom waters;
- Mechanical or chemical disruption by treatment (Peterson et al. 1995, Velzeboer et al. 1995, Durrer et al. 1999, Jüttner and Watson 2007).

An ever-increasing list of cyanobacteria species are now formally or tentatively identified as geosmin and/or MIB producers (e.g., Watson 2003). A valuable pictorial listing of some major producers in US surface waters was recently published by Izaguirre (2007). Advances in genetics and biochemistry have provided a new ability to identify closely related species that may differ morphologically and/or are highly plastic in cell, filament, and colony morphology (e.g., Anagnostidis and Komárek 1988). These methods may also provide insight into the biosynthetic pathways and genetic/environmental control of VOC production, which have been well characterized for geosmin production by *Streptomyces* but are still unresolved among cyanobacteria (e.g., Spiteller et al. 2002, Jiang et al. 2006, Jüttner and Watson 2007). Nevertheless, the continually evolving taxonomic classification of these organisms—previously thought to be algae—can create problems with cross-referencing to species described by their traditional names. For example, many of the taxa collectively grouped under the genus *Oscillatoria* have been reassigned to new and existing genera (e.g., *Geitlerinema, Tychonema, Planktothrix, Limnothrix, Phormidium, Oscillatoria*; see Watson 2003). A survey of those taxa known to produce geosmin, MIB, and other VOCs is provided in Table 15-3, listed under their revised names, along with the earlier synonyms.

Both geosmin and MIB are produced by filamentous and coccoid cyanobacteria (planktonic and benthic), and a few species produce both compounds. Planktonic cyanobacteria have long been implicated in geosmin and MIB production (e.g., Wnorowski 1992, Persson 1996), but in fact both planktonic and benthic producers may be present in a water body and act together, in succession, or covary over a season (Sugiura et al. 1998, Ridal et al. 2007, Watson et al. 2007a). Benthic, shoreline, and periphytic* populations can be significant odor sources but are often overlooked and difficult to trace (Izaguirre 1992, Izaguirre and Taylor 1995, Watson and Ridal 2004). It is also of note that a number of picocyanobacterial geosmin and MIB producers (*Synechococcus* spp.) have been isolated (Izaguirre 2007); the potential role of these minute (~ <1–2 μm) cells in T&O events has long been overlooked (see Size Fractions section) and needs further investigation. Many planktonic cyanobacteria control their buoyancy in response to light, which can lead to significant and rapid vertical and horizontal changes in biomass and VOCs. Deep-living populations may migrate to the surface overnight and appear as dense surface scums, which can be moved toward shorelines by wind or water movement. Such highly patchy and dynamic spatial distributions are particularly challenging to monitor (Watson et al. 2007a).

Geosmin and MIB concentrations vary widely in natural systems. In planktonic environments, geosmin and MIB levels typically range from <1 ng up to ~0.5–1 μg/L (e.g., Jüttner et al. 1986, Jones and Korth 1995, Davies et al. 2004, Watson et al. 2007a). In more extreme cases, far higher concentrations are achieved by massive population die-off; for example ~250 μg/mL geosmin was produced by a dying surface bloom of *Gloeotrichia echinulata* following a tornado-driven event in a small stratified

* That is, growing on a living or inert substrate.

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Table 15-3 Planktonic (P), benthic (B), or epiphytic (E) cyanobacteria identified as VOC producers (G = geosmin, M = 2-methylisoborneol, β = β-cyclocitral; DMD = dimethyl disulfide; DMT= dimethyl trisulfide; DID = diisopropyl disulfide; DIT = diisopropyl trisulfide). Where >1 compound has been identified, the dominant VOC (as measured by production rate, not OTC) underscored, if known. (Source: Adapted from Watson 2003.)

Species	Synonym	VOC	HAB
Anabaena circinalis		G[2]	PL
Anabaena crassa		G[6,1]	PL
Anabaena laxa (CA783)		G[1]	PL
Anabaena lemmermannii		G[18,1]	PL
Anabaena macrospora		G[17]	PL
Anabaena scheremetievi		G[1]	PL
Anabaena solitaria		G[12]	PL
Anabaena viguieri		G[19]	PL
Aphanizomenon flos-aquae		G[5]	PL
Aphanizomenon gracile		G[5]	PL
Geitlerenema splendidum	*Oscillatoria splendida*	G[16]	B
Gloeotrichia echinulata		G	B / PL
Hyella fontana (4 strains)		MIB, G[1]	E
Jaaginema geminatum	*Oscillatoria geminata*	MIB[17]	B
Leibleinia sp.	*Lyngbya subtilis*	G[14,1]	B
Lyngbya aestuarii		MIB[16]	B
cf. *Microcoleus* sp.		G[8]	PL
Microcystis aeruginosa		β, DMD, DMT[4]	PL
Microcystis flos-aquae		β, DID, DIT[4]	PL
Microcystis botrys		β[4]	PL
Microcystis viridis		β[4]	B
Microcystis wesenbergii		β, DMD, DMT[4]	PL
Nostoc sp.		G[1]	B
Oscillatoria curviceps		MIB[7,1]	B
Oscillatoria limosa (strain specific)		MIB[8,1]	B
Oscillatoria simplicissima		G[1]	B
Geitlerinema splendidum	*Oscillatoria splendida*	G[1]	B
Oscillatoria tenuis v. levis		MIB[7]	B
Oscillatoria variabilis		MIB[16]	B
Phormidium allorgei	*Lyngbya allorgei*	G[15]	B
Phormidium amoenum	*Oscillatoria amoena*	G[17]	B
Phormidium breve (strain specific)	*Oscillatoria brevis*	G, MIB[10]	B
Phormidium calcicola		MIB,G[1]	B

Table continued next page

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Table 15-3 Planktonic (P), benthic (B), or epiphytic (E) cyanobacteria identified as VOC producers (continued)

Species	Synonym	VOC	HAB
Phormidium chalybeum	*Oscillatoria chalybea*	MIB[13]	B
Phormidium cortianum	*Oscillatoria cortiana*	G[16]	B
Phormidium formosum	*Oscillatoria formosa*	G[12]	B
Phormidium favosum		MIB[15]	B
Phormidium cf. *inundatum* (LO584)		G[1]	B
Phormidium simplissimum	*Oscillatoria simplicissima*	G[12]	B
Phormidium tenue	*Oscillatoria tenuis*	MIB[12]	B
Phormidium uncinatum		G[15]	B
Phormidium viscosum		G[15]	B
Phormidium LM689		MIB[20]	B
Phormidium sp. NIVA CYA 51		G, MIB[3]	B
Planktothrix aghardhii (strain specific)	*Oscillatoria aghardhii*	G, MIB (tr)[3,12]	PL
Planktothrix cryptovaginata	*Lyngbya cryptovaginata*	MIB[12]	B
Planktothrix prolifica	*Oscillatoria prolifica*	G[10]	B
Porphyrosiphon martensianus	*Lyngbya martensiana*	MIB[8]	B
Pseudanabaena catenata		G, MIB[9]	PL
Pseudanabaena limnetica	*Oscillatoria limnetica*	MIB[17]	PL
Schizothrix muellerii (actinomycete contaminant)		G[10]	B
Symploca muscorum		G[16]	SOIL
Synechococcus sp. (LP1181)		MIB[1]	PL
Synechococcus sp. (LP1182)		MIB[1]	PL
Synechococcus sp. (CL792)		MIB,G[1]	PL
Synechococcus sp. (BCR1092)		MIB[1]	PL
Synechococcus sp. (BPP1000a,b)		MIB,G[1]	PL
Tychonema bornetii (strain specific)	*Oscillatoria bornetii*	G[3]	B
Tychonema granulatum	*Oscillatoria* f. *granulata*	G, MIB[17]	B

[1]Izaguirre (2007); [2]Bowmer et al. (1992); Rosen et al. (1992) ; [3]Berglind et al. (1983); [4]Jüttner (1984); Hofbauer and Jüttner (1988); [5]Jüttner et al. (1986) ; [6]Izaguirre, personal communication; [7]Izaguirre et al. (1983) ; [8]Izaguirre and Taylor (1995); [9]Izaguirre et al. (1999); [10]Naes et al. (1988); [11]Persson (1983); [12]Persson (1988); [13]van der Ploeg et al. (1995); [14]Schrader and Blevins (1993); [15]Sugiura et al. (1998); [16]Tabachek and Yurkowski (1976); [17]Tsuchiya and Matsumoto (1988); [18]Watson and Izaguirre unpublished data; [19]Wu et al. (1991); [20]Zimmerman et al. (1995); [21] Wu (1988).

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prairie lake (Watson 2004).* Periphyton can produce in the order of 0.1–1.0 μg/cm^2 or 100 ng/mg dry wt (Matsumoto and Tsuchiya 1988, Watson and Ridal 2004); benthic mats up to 1,000 ng/mg dry wt (Vilalta et al. 2004); levels of between ~0.5–12 μg/kg have been measured in soil (Stahl and Parkin 1994). Mixed cyanobacterial/fungal/bacterial populations in secondary treatment units can also develop substantial levels of geosmin and MIB (e.g., 1,600–128,000 ng/L, 10–50 ng/L, respectively; Watson et al. 2003).

Cyanobacterial-derived geosmin and MIB have been almost exclusively attributed to aquatic sources. However, terrestrial sources can make significant contributions to VOC levels in the receiving waters, for example spring melt or storm events (Hrudey et al. 1992). In most cases this has been related to increased actinomycete levels in the water (e.g., Jensen et al. 1994, Zaitlin and Watson 2006; see Nonalgal Biological Odor Moderators and Sources section); however, odor-producing cyanobacteria (e.g., *Hyella, Nostoc, Phormidium*) and microbiota (fungi and molds such as *Penicillium*, myxobacteria, etc.) are common to terrestrial and wetland environments, and potentially, cells and/or VOCs could also be introduced in this manner. To date, these terrestrial population distributions, and their potential contribution to aquatic odor, are largely unmeasured.

Floral/fruity/tobacco odors: nor-carotenoids. Nor-carotenoids are an important group of algal isoprenoids that are produced during the oxidation of carotenes and function in many terrestrial plant–animal chemical interactions. Pigments such as α- and β-carotene, zeaxanthin, and xanthophyll are common among photosynthetic taxa, and derivatives of these carotenes are produced by most algal phyla. As seen with geosmin and MIB, many nor-carotenoids have molecular enantiomers, and the naturally produced isomers are usually more potent (and likely more biologically active) than the synthetic molecules (see Theimer et al. 1977). Some of these compounds have strong odors (hay, tobacco, floral, or fruity) and low OTCs and can contribute toward aquatic odor—although not with the same widespread impacts as geosmin and MIB (Jüttner 1981a, Jüttner and Hans 1986, Chorus et al. 1990; Table 15-2). Carotene oxidase products have been found to have potentially beneficial or detrimental effects in humans (see Carail and Caris-Veyrat 2006), and evidence suggests that some of these compounds found in aquatic systems (e.g., β-cyclocitral, β-ionone, 6-methyl-5-hepten-2-one, hexahydro farnesyl acetone, geraniol, and dihydroactnidiolide) also have semiochemical†/antimicrobial properties (see Algal Taxa section).

Production occurs as a result of changes in cell pigments and can increase during cell breakdown as a result of heightened oxidative enzymatic activity (involving specific enzymes such as carotene oxidase; Jüttner and Höflacher 1985). Nor-carotenoids such as 6-methylhept-5-en-2-one, dihydroactinidiolide, β-ionone, and α- and β-cyclocitral have been identified from both field (lake, reservoir, river) and culture samples (Figure 15-3; e.g., Jüttner 1981a, 1984, 1992; Henatsch and Jüttner 1983; Chorus et al. 1990; Jüttner 1995; Rashash et al. 1996; Table 15-1). Aquatic levels of these nor-carotenoids are often correlated with the pigment content an algal community, which may change rapidly in response to short- and long-term taxonomic and environmental shifts (e.g., Jüttner et al. 1986, Soma et al. 1993).

With the notable exception of β-cyclocitral (below), aquatic concentrations of most nor-carotenoids range below 50 ng/L (Jüttner et al. 1986). In many cases, these compounds are not diagnosed as the primary sources of odor, and it is likely that they contribute to (rather than dominate) a VOC mix that affects the overall T&O (Jüttner 1995, Höckelmann and Jüttner 2004). In fact, "blended olfactory signals" may play an

* Pine Lake, Alta., Canada; this T&O event was entirely unanticipated because this eutrophic lake had been subject to attempted remediation via hypolimnetic water removal, and planktonic blooms reduced.

† That is, biological activity, e.g., as grazer deterrents, toxins, inhibitors, pheromones, etc.

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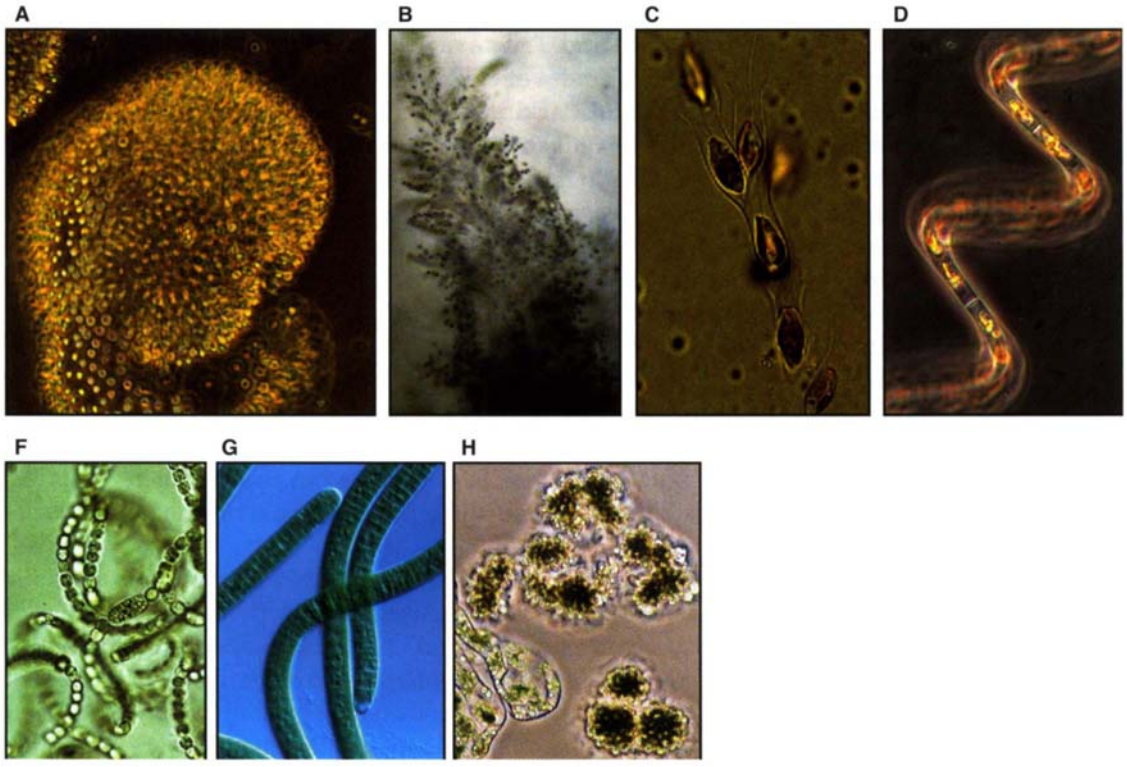

Figure 15-4 Taste and odor producing algae. Oxylin producers: (A) *Uroglena americana*, (B) *Hydrurus foetidus*, (C) *Dinobryon cylindricum*, (D) *Synura petersenii* (E) *Aulacoseira granulata*; (F) geosmin producer, *Anabaena lemmermannii*; (G) MIB producer, *Oscillatoria* cf. *curviceps* (Courtesy of G. Izaguirre); (H) β-cyclocitral producer, *Microcystis* spp.

important role as attractants and repellents in foraging planktonic and benthic grazers (Höckelmann et al. 2004, Fink et al. 2006).

β-cyclocitral is an important flavor compound derived from the oxidative cleavage of β-carotene, a process triggered by cell damage or death. This nor-carotenoid has been found to be responsible for an offensive tobacco odor during T&O events and is more frequently reported from surface waters than most other nor-carotenoids. β-cyclocitral typically occurs in eutrophic systems at ng/L to μg/L levels (Jüttner et al. 1986, Chorus et al. 1990) but may reach ~0.75 mg/L in ephemeral windblown surface scums (Watson 2003, 2004). In particular, β-cyclocitral is a major volatile compound of the common hepatotoxic genus *Microcystis* (Figure 15-4). All species of *Microcystis* tested to date (toxic and nontoxic) liberate significant quantities of this compound, which may function as a grazer deterrent (Jüttner 1981a, Jüttner and Höflacher 1985, Watson et al. 2007b). At trace levels, β-cyclocitral may contribute to floral odors, along with α- and β-ionones; other important and generally more potent nor-carotenoids produced cyanobacteria and other taxa (Tables 15-2 and 15-3).

Oily/Fishy/Floral/Vegetable Odors: Fatty Acids and Oxylipins

Fatty acids. Cell fatty acid (FA) chemistry divides algae into two general groups and also influences the major VOCs produced by these taxa. Cyanobacteria and Chlorophyta have a relatively low FA content, mainly consisting of saturated and monounsaturated FAs ($\leq C_{18}$) and some polyunsaturated fatty acids (PUFAs). Chrysophyta, Haptophyta, Cryptophyta, and Dinophyta have a high cell content of unsaturated and polyunsaturated FAs that serve as a major form of energy storage. Many of these algal

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taxa are rich in ϖ-3 and ϖ-6 PUFAs of up to C_{22} or higher (e.g., α- linolenic, docosahexenoic [DHA], and eicosapentaenoic acid [EPA]), which are important sources of volatile PUFA-derived metabolites (oxylipins).

Intact PUFAs are not a major source of T&O. Some have mild but identifiable odors (Table 15-2), but most are virtually odorless, and high concentrations are not commonly found in aquatic environments. They are highly insoluble in water, and under most natural field conditions are broken down rapidly by heterotrophic activity (Chrost and Gajewski 1995). In fact, many PUFAs are highly toxic when present in free form (Jüttner 2001), and in healthy cells, they are incorporated into stable components such as thylakoid* and other membrane structures. In a few cases, some of the more common PUFAs have been reported in water and culture material; for example, cyanobacterial and green algal cultures (e.g., linolenic acid; Rashash et al. 1996) and oily field slicks of reddish-orange *Botryococcus braunii,* a large, colonial chlorophyte with an unusually high cell lipid content (Wake and Hillen 1981, Chiang et al. 2004). However, "free intracellular PUFAs" can be generated as artifacts by most current analytical techniques (Jüttner 2001), and there is no evidence to show that these are liberated by cells in any appreciable amount during growth. It is of note, however, that significant levels of rank-smelling short-chain FAs (e.g., butryric, valeric, and isovaleric) and associated esters can be produced by bacterial degradation of algal and nonalgal organic material. This process can contribute to odor levels in source waters and treatment plant filter beds (e.g., Karlsson et al. 1995).

Oxylipins: production and properties. Unlike FAs precursors, oxylipins are a notorious source of rancid, oily, fishy, cucumber, fruity, and floral odors in drinking water supplies, essential oils, and spoiled lipid-rich foods (Eriksson et al. 1976, Karahadian and Lindsay 1989, Whitfield 1998, Watson et al. 2001a). It is important to understand how, when, and why oxylipins are produced, since this has considerable influence on the timing and severity of T&O events.

As with PUFAs, many oxylipins are highly bioactive and not produced by healthy growing cells in any quantifiable amounts. Their production is triggered by the loss of cell integrity as a result of senescence, rapid environmental changes (salinity, temperature, desiccation, etc.), and agents such as grazers, parasites, or water treatment. Cell damage/death instantaneously activates catalytic enzymes such as lipases and lipoxygenases (LOX), producing a variety of oxylipins that include dienes, dienals, and trienes, together with related alcohols, alkanes, alkenes, aldehydes, ketones, and hydrocarbons (e.g., Jüttner 1995, Fontana et al. 2007).

Odorous oxylipins vary considerably in stability and potency (OTCs range between ~0.08 and 300 µg/L; Table 15-1). The majority can periodically contribute to the "olfactory blend" of the water, but only a few are major sources of T&O. In particular, the polyunsaturated aldehydes (PUAs) 2,4-heptadienal, 2,6-nonadienal, 2,4-decadienal, and 2,4,7-decatrienal are most frequently implicated in odor events, often described as "rancid/fishy/oily/cucumber" (e.g., Yano et al. 1988; Burlingame et al. 1992; Jüttner 1981b; Jones and Korth 1995; Watson et al. 2001a, 2001b). Other oxylipins are also implicated on occasion: 4-heptenal, n-pentanal, and n-heptanal have been linked to fishy odors; n-hexenal and "leaf alcohols" (e.g., 3-hexenol) linked to grassy odors (as well as the nor-carotenoid β-cyclocitral, above) (Jüttner et al. 1986, Khiari et al. 1995, Cotsaris et al. 1995; Tables 15-1 and 15-2).

As noted above, complete analytical quantification must include all major molecular isomers, and this is particularly relevant for unsaturated oxylipins. Oxidative PUFA cleavage yields *trans, cis* unsaturated derivatives, but subsequent (photo- or thermal) isomerization produces a mixture of *trans, cis,* and *trans, trans* isomers (e.g.,

* Specialized cell membrane structures that are the site of photosynthesis.

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Yano et al. 1988). These processes also have important implications for sensory odor assessment, since isomers may have different OTCs and odors. For example, *trans, cis,* and *trans, trans* 2,4-decadienal have OTCs* of 20 μg/L and 320 μg/L, respectively. *Trans, cis,* and *trans, trans* 2,6-nonadienal have "grassy/cucumber" and "oily, tallow" odors; *trans, cis, cis*-2,4,7-decatrienal has a "fishy/cod-liver oil" odor; and *trans, trans, cis* 2,4,7-decatrienal, a "green/cucumber/melon" smell (Meijboom and Stroink 1972, Burlingame et al. 1992).

Oxylipins: algal sources. Although odorous unsaturated oxylipins are produced by most algal groups (Table 15-2), lipid-rich taxa are the major sources. Chrysophytes are particularly renowned for rancid/fishy/oily smelling C_7, C_9, and C_{10} polyunsaturated aldehydes in a range of surface waters, including oligotrophic and remediated systems (Figure 15-4; Jüttner 1995; Jones and Korth 1995; Rashash et al. 1995; Yano et al. 1988; Watson et al. 2001a, 2001b). Diatoms are also frequently implicated in source water T&O, although their VOC chemistry has been characterized for relatively few freshwater species. Early sensory reports described moderate abundances of planktonic diatoms (*Synedra, Fragilaria, Cyclotella, Asterionella, Stephanodiscus, Aulacoseira, Tabellaria*) as "grassy/fruity/floral," and high densities as "fishy" (Mallevialle and Suffet 1987), which might suggest variable concentrations of VOCs such as n-heptanal. A few analytical studies have reported fishy/fatty/grassy-smelling VOCs such as 2,4-octadienal, 2,4-heptadienal, octanal, 4-heptenal, and heptanal in some planktonic and benthic diatoms (*Aulacoseira granulata* [Figure 15-4E], *Melosira varians, Fragilaria* sp., *Stephanodiscus hantzschii,* and *Synedra delicatissima*; Table 15-1). Other diatoms produce different oxylipins, some of which were first identified among marine pheophytes (brown algae) as pheromones. *Asterionella formosa*, a common planktonic diatom, can have an intense geranium odor, generated by the production of fucoserratene via catalytic PUFA oxidation (*trans, cis*-1,3,5-octatriene), together with octadienes (Jüttner and Müller 1979; Table 15-1). Conversely, this species has not been found to produce the C_7–C_9 unsaturated aldehydes (2,4-heptadienal, 2-4-decadienal, 2,4,7-dectrienal, 2,6 nonadienal). Fucoserratene production is not unique to *Asterionella formosa*; this compound has been identified during a bloom of *Synura uvella*† and in cultures of the cyanobacteria *Anabaena oscillarioides* (together with other C_8 hydrocarbons, 2,4-heptadienal, and other VOCs not detected in *Asterionella*). Some periphytic diatom species produce alicyclic PUFA derivatives such as Ectocarpene ("tomato-leaf like" odor) and Dictyopterenes A and C' ("seaweed" odor), which are released in pulses over the growing season (Wendel and Jüttner 1996, Jüttner and Durst 1997; Table 15-2). These compounds have not been identified in planktonic diatom species.

As noted above, Chrysophyceae and Synurophyceae are notorious sources of fishy-smelling C_7, C_9, and C_{10} PUAs and other odorous oxylipins; however, most T&O events involving this group have been attributed to a select few species of large colonial flagellates, notably *Dinobryon cylindricum, D. divergens, Uroglena americana, Synura petersenii,* and *S. uvella* (e.g., Jüttner 1981b; Wee et al. 1994; Watson et al. 1999a, 2001a, 2001b). The unicellular flagellate *Ochromonas* is also commonly implicated; however, this can often be mistakenly identified since it is morphologically similar to single cells of *Uroglena.*‡

These species each produce several different PUAs in differing amounts, which change over cell growth and with different environmental conditions (Watson and

* In paraffin oil.

† Note that A. formosa was also present in the plankton at low abundance.

‡ After colony disintegration; particularly problematic with stored fresh samples or those preserved with formaldehyde and Lugols iodine.

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Satchwill 2003). A few patterns have been identified: high levels of 2,4-heptadienal are often traced to *Uroglena*; 2,4-decadienal and 2,4,7-decatrienal are commonly observed during outbreaks of *Dinobryon* and *Synura*. Of interest is the production of the unconjugated C_9 aldehyde 2,6-nonadienal, originally thought to be diagnostic of a single species of *Synura* (*S. petersenii;* Figure 15-4D), and of a specific enzyme cleavage pattern* (see Wee et al. 1994, Jüttner 1995). This species is a notorious source of 2,6-nonadienal, but recent work has now identified production in at least two synurophytes (*S. petersenii, S. uvella*), mixed periphyton communities and *Hydrurus* mats; Watson, Jüttner and Wee; unpublished).

Other taxonomic groups (Haptophyta, Xanthophyceae, Cryptophyta, Dinophyta, cyanobacteria, and Chlorophyta) are reported on occasion to produce similar odors (e.g., Mallevialle and Suffet 1987, Rashash et al. 1995, Watson 1999). Oxylipins have been reported from species belonging to some of these groups (*Cryptomonas rostratiformis, Peridinium willei, Scenedesmus subspicatus, Microcystis aeruginosa*; Cotsaris et al. 1995, Rashash et al. 1996, Watson et al. 1998), but others (e.g., *Chrysochromulina, Tribonema, Gymnodinium, Eudorina, Chlamydomonas, Gonium, Volvox*) have not been chemically characterized to date.

Physiological ecology and "hidden" oxylipin sources. Environment, taxonomy, and physiology all modify VOC production; and similar to other VOCs (e.g., terpenoids), there are significant differences in oxylipin chemistry and production capacity among species, and even among strains of the same species. Genetics, age, nutritional mode (heterotrophic vs. autotrophic), and environmental factors control and modulate innate production capacity (i.e., inducible through cell lysis). As shown for terpenoids, oxylipin production varies both among and within species. For example, PUA production was not detected for certain species or strains of the genera *Uroglena, Dinobryon, Synura, Mallomonas, Synedra, Stephanodiscus, Fragilaria,* and *Aulacoseira* (Watson and Jüttner unpublished), and 23 of 62 marine diatom strains surveyed (Wichard et al. 2005). However, sampling regime and timing and/or culture conditions can produce misleading results: PUA yield and chemistry vary significant over growth and with environment (Wee et al. 1994, Watson and Satchwill 2003). Light, temperature, and nutrients affect cell FA composition (e.g., Napolitano 1994, Lynn et al. 2000) and the rate and timing of release, which are also increased by irradiance, heavy metals, chlorine, and pesticides (e.g., Jüttner 1995). For example, the freshwater diatom *A. formosa* shows elevated lipid storage and oxylipin production with silica limitation (Chorus et al. 1990).

In many cases, the biological source of oxylipins may not be manifested as visible blooms. Chrysophyceae such as *Dinobryon*, *Uroglena,* and *Chrysosphaerella* are mixotrophic flagellates† and can produce T&O in moderately polluted water bodies, especially where there are elevated organic or bacterial levels. These colonial flagellates can develop "hidden" populations in low-light deep or under-ice zones that are enriched in bacteria and detritus (Nicholls 1995, Watson et al. 2001a). As with buoyancy-controlling cyanobacteria, some of these species show significant diurnal vertical movement through the water column—an important consideration for sampling protocol and intake pipes (see Yano et al. 1988). Other overlooked potential sources include ice communities, which can be rich in diatoms trapped at freeze-up, as is seen,

* The "heterolytic" cleavage pathway for the production of unconjugated oxylipins (cf. Gardiner 1991; Biochem. Biophys. Acta 1084:221–39) is now disputed.

† These can derive nutrients and energy from both photosynthetic and organic sources (dissolved organic matter, bacteria, other algae).

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for example, in lakes Winnipeg and Erie (H. Kling,* unpublished, S. Wilhelm,† personal communication).

Upstream sources should not be overlooked as a source of oxylipins and other VOCs. Dense beds of attached algae can release high VOC levels, generated through the disruptive activity of grazers and physical processes, both *in situ* and as detached mats. A notable example is *Hydrurus foetidus* (Figure 15-4B), a multicellular chrysophyte that grows in cold, fast-flowing waters as attached, frondlike masses, which can release extremely high levels of rank fetid-smelling PUAs, as its name suggests (Watson 2003). Algal mats and other aquatic plants are often colonized by epiphytic algal communities that are rich in diatoms which can produce VOCs. Similarly, river and shoreline biofilms are often dominated by diatoms, and can represent significant sources of oxylipins liberated by grazers and mechanical disruption such as scouring (Jüttner and Dürst 1997, Jüttner 2001, Watson 2003). It is of note that these biofilms can also colonize other surfaces, including intake and distribution pipes, and generate significant VOC levels (Skjevrak et al. 2005).

Nitrogen-containing VOCs

The potential role of nitrogen-containing algal metabolites in T&O has not been investigated, yet there is intriguing evidence to suggest that these may be a source of odor. For example, there are unsolved fishy odor outbreaks where oxylipins are absent and the odor compound(s) not easily extracted by conventional stripping methods (see Khiari et al. 1995). Many amines have highly potent fishy or putrid odors and low OTCs (Table 15-2), but they are not often measured, since they are not well recovered by many analytical protocols routinely used for T&O compounds. Amines can be formed through bacterial decarboxylation of amino acids from plant and animal material, or as treatment by-products, in source waters and treatment plants/distribution systems (Mallevialle and Suffet 1987), and the occurrence of these compounds in raw and treated waters is generally attributed to these processes. Amines and other N-compounds are also produced by living algae, a potential source that has been generally disregarded. In particular, a number of potent amines (diethylamine, trimethyl-amine, 1-aminopropane, and ethanolamine) have been detected in significant levels (ca. 50–300 µg/L) in cultures of cyanobacteria, Chlorophyta, and Chrysophyta (Herrmann and Jüttner 1977, Pohnert and von Elert 2000), and in at least one case, there was indisputable evidence that these compounds were of algal, not bacterial, origin. Amines were also detected in water collected from a *Chlamydomonas* bloom in the Ruhr River (Germany) and cultures derived from this bloom (Herrmann and Jüttner 1977).

A diversity of other volatile N-compounds (heterocyclic compounds, aromatics, alkoxyamines, and amides) have been reported from (nonaxenic) strains of the cyanobacteria *Aphanizomenon flos-aquae* (Dembitsky et al. 2000). These VOCs have similarly not been investigated as sources of T&O. *A. flos-aquae* and related species are particularly problematic in numerous small and large eutrophic and mesoeutrophic lakes (e.g., Lin et al. 1979, Peterson et al. 1995). While *A. flos-aquae* does not produce geosmin‡ or MIB, this species can be a source of alkaloid neurotoxins (see Chapter 3) and is commonly associated with "mouldy, grassy, vile" source water odors.

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‡ Although strong geosmin production has been reported in a cyanobacteria identified as *A. gracile*; Jüttner 1984; Durrer et al. 1999.

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Fermentation Products and Biogenic Sulfides: Putrid/Rotten/Swampy/Sulfurous Odors

It has been known for some time that some cyanobacteria and algae synthesize volatile fermentation products during cell growth (Jüttner 1995) and impart odor to water. These VOCs include alcohols, esters, and alkyl sulfides, which also contribute to the septic and putrid odors produced during bacterial degradation of algae and plant material (Cotsaris et al. 1995, Jones and Korth 1995). Dimethyl disulfide, dimethyl trisulfide, and 3-methyl butanal are especially potent organic volatile sulfur compounds (VSCs) produced by bacterial activity (Wnorowski and Scott 1992, Cotsaris et al. 1995, Karlsson et al. 1995), and open water levels of most of these compounds often originate from sediment surfaces, rotting shoreline algal and plant material, or spring runoff. And in fact, VSCs reported from inland waters and algal cultures are generally considered products of bacterial degradation (Bechard and Rayburn 1979, Richards et al. 1991). Nevertheless, some algae are significant sources of these compounds, which are produced as secondary metabolites during growth. In particular, species of the Characeae (stoneworts) are often called "stinkweed." Members of this group (*Chara aspera, C. globularis,* and *Nitellopsis obtusa*) produce highly volatile thiols (4-methylthio-1,2-dithiolane and particularly 5-methylthio-1,2,3-trithiane) together with several potent nor-carotenoids (e.g., Anthoni et al. 1980, Bankova et al. 2001, Berger and Schagerl 2004). These macroalgae can form dense submerged beds in a range of shallow waters, but it is not known if they are significant sources of T&O.

Some cyanobacteria can also produce significant levels of VSCs during growth. Field populations and cultures of *Microcystis (M. aeruginosa, M. wesenbergii, M. flos-aquae)* show strain-specific production of potent thiols (isopropyl mercaptan, isopropyl disulfide, methyl isothiocyanate, isopropyl methyl sulfide, and isopropyl methyl disulfide; Hofbauer and Jüttner 1988). Because of their high volatility, these compounds can produce significant airborne odors but do not appear to affect most treated drinking water supplies, as they are removed by chlorination (although this can produce chlorinated by-products; Tsuchiya et al. 1992).

ALGAL TAXA

General Trends

Some general differences in the organoleptic profiles of major algal taxonomic groups can be recognized (Table 15-2), characterized by distinct odor types, patterns in biosynthesis and timing of their release, and effects on source waters:

1. Terpenoids are perhaps the most important group of T&O compounds produced by cyanobacteria, notably geosmin, MIB, and nor-carotenoids. These are relatively stable, resist conventional treatment, and can persist in the open water or be translocated by water movement. Many terpenoids are produced over growth but stored in intracellular pools that can show a delayed release with cell breakdown.

2. Chrysophytes, synurophytes, and diatoms have a higher PUFA cell content, and fishy/oily/floral/cucumber oxylipins derived from these cell components are the most significant group of VOCs produced by these groups, notably 4-heptenal, 2,4-heptadienal, 2,4-octadienal, 2,4,7-octatriene, 2,6 nonadienal, 2,4-decadienal, and 2,4,7-decatrienal. Production is triggered by cell lysis and typically occurs at the end of blooms or with mechanical or physiological cell disruption. Many of these compounds are highly reactive and short-lived but show differences in stability and treatment responses.

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3. Chlorophyta (green algae) are most commonly associated with "grassy" odors characterized by *cis*-3-hexanol or green leaf alcohol, also an oxylipin. This taxonomic group includes a select group of the macroalgae (Charales; "stinkweed") that produce potent thiols.
4. Other algal groups have been less well characterized, although both cryptophytes and dinoflagellates produce oxylipins and other hydrocarbons and are sometimes described as "floral" or "fishy."

While none of the major VOCs is produced by all algal taxa, most are produced by more than one species from the same or different groups (Table 15-1). As noted above, evidence to date suggests that no single algal group or species has a unique "organoleptic fingerprint," although select groups of species are known for their high production rates of some VOCs (e.g., β-cyclocitral, oxylipins such as fucoserratene and 2,6-nonadienal, and thiols), which can provide valuable diagnostic signals. Again it must be emphasized that the majority of algal taxa have not been characterized for VOC production, and research is increasingly pointing toward "hidden" biota as important sources of T&O.

This general lack of VOC specialization by most species is in part because many VOC precursors and synthetic pathways are common to a number of taxa (above). As noted, for a given species, the production of a particular VOC may differ among laboratory clones or spatially/temporally distinct populations, which may reflect genetics, short-term changes in environmental conditions, or the development of different ecotypes. It may also reflect the fact that relatively few studies have used axenic cultures (Persson and Jüttner 1983).

Humans are not the only organisms that perceive and respond to odor compounds—which, by definition, are biologically active. It is now known that many T&O compounds are produced as important chemical signals (semiochemicals) that modify cell functioning (e.g., antioxidants, pheromones, autoregulation) and food web interactions (grazer deterrents, inhibitors, toxins, attractants). This is an intriguing and growing area of research (e.g., see Schauenstein et al. 1977; Jüttner 1979, 1981a, 2001; Kamiya et al. 1978; Anthoni et al. 1980; Rechardt 1981; Höckelmann et al. 2004; Chiang et al. 2004; Wichard et al. 2005; Fink et al. 2006; Watson et al. 2007b; also the review by Watson 2003 and references therein). Understanding the function of these VOCs—*who* produces them and *why*—may go a long way toward our ability to predict their occurrence and levels in source waters—that is, *when* and *where* they are produced.

Size Fractions

Surface water T&O is commonly attributed to blooms of netplankton such as filamentous and colonial cyanobacteria, chrysoflagellates, and diatoms or large thecate dinoflagellates. High densities of smaller taxa (nanoplankton) are also known to produce significant odor events, such as *Chrysochromulina breviturrata* and *Stephanodiscus parvus* (Nicholls 1982, Watson et al. unpublished). Picoplankton are minute (<2 μm) photosynthetic cyanobacteria and eukaryotes (e.g., chlorococcales) that are common to many meso- or oligotrophic systems and highly eutrophic lakes (Carrick and Schelske 1997). To date, these cells have been overlooked in odor studies, which have focussed on the more conspicuous net- and nanoplankton. These and other algal sources (e.g., benthic, littoral, lentic, terrestrial) need to be reassessed as a potential source of odor. Cyanobacterial picoplankton ("picocyanobacteria"), notably *Synechococcus*, have been shown to produce a number of potent VOCs, including nor-carotenoids and the terpenoids geosmin and MIB, although as with other taxa, production varies among strains

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and species (Izaguirre et al. 1982, Henatsch and Jüttner 1983, Izaguirre 2007). Henatsch and Jüttner (1983) noted that the predominant odor produced by cultures of *Synechococcus* was pleasant, as a result of their production of a number of nor-carotenoids with flora/fruity odors (see Floral/Fruity/Tobacco Odors: Nor-carotenoids section; Table 15-4). To date, there are no published odor studies of other picoplankton groups, but these are predominantly chlorophytes (*Nannochloris, Coccomyxa* etc.), a group that is usually associated with less pleasant "green" odors.

Taste and Odor and Toxins

Cyanobacteria can not only impair source water with T&O but also through the production of one or more toxins that present a risk to humans or other organisms (Chapter 16). Similar to VOCs, there are a large number of cyanobacterial toxins that have now been identified. These toxins are difficult to detect and predict—a major concern with water providers and managers, many of whom lack the resources to assess the potential risk(s) associated with cyanobacterial outbreaks. The risk of these toxins cannot be determined by simple visual inspection of a water body, and in cases where toxins cannot be analyzed directly, risk is often assessed using surrogate measures such as cell counts and T&O. However, both of these measures can be misleading, for several reasons. (1) Not all blooms are produced by cyanobacteria, and color is no indication of the presence of these taxa, which show a variety of shades of red, green, blue-green, and yellow. (2) As seen with VOCs, per capita production of different toxins varies considerably both within and among cyanobacterial species; strains of the same species can include producers and nonproducers. The presence of cyanobacteria in a bloom and/or abundance measures of these taxa therefore will not necessarily be correlated with toxin levels. (3) Toxins are colorless and tasteless, thus do not themselves contribute to T&O; furthermore, there are no known direct physiological links between odor and toxin production. Thus in many cases, outbreaks of T&O are not concurrent with (or caused by) toxic biota, as shown in a recent survey of these two types of impairments in the Great Lakes (Watson et al. 2008).

It is therefore of vital importance to stress that *not all odor-causing blooms are toxic, not all toxic blooms are odorous, and both toxins and odor can occur in the absence of visible blooms.*

NONALGAL BIOLOGICAL ODOR MODERATORS AND SOURCES

While this chapter is devoted to algae and cyanobacteria as sources of T&O, it is important to recognize the effects of other biota on the production and fate of VOCs in surface waters. A number of bacteria, zooplankton, fungi, and bryophytes are known to produce and/or metabolize odor compounds (Jüttner and Hans 1986, Chrost and Gajewski 1995, Izaguirre 2007, Saadoun et al. 1997, Saadoun and El 1998, Durrer et al. 1999, Saito et al. 1999). In particular, some actinomycetes (the filamentous bacteria *Streptomyces*) have long been thought to be major sources of geosmin and MIB in aquatic systems. However, it is now evident that where photosynthetic growth is supported, cyanobacteria are the most important sources of these compounds (see, e.g., reviews by Zaitlin and Watson 2006, and Jüttner and Watson 2007). Most actinobacteria are widespread terrestrial aerobes. These and other soil biota (e.g., molds such as *Penicillium*, other fungi and bacteria [e.g., *Nocardia*], and cyanobacteria) may increase geosmin and MIB levels when washed in from watersheds (Persson and Sivonen 1979, Jensen et al. 1994, Zaitlin et al. 2003). However, it is not known to what extent these terrestrial organisms continue active growth and geosmin or MIB production in surface waters, although there is evidence of *in situ* production by some species that are indigenous to aquatic environments (Klausen et al. 2004). These actinobacteria, and

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Table 15-4 Odor compounds produced by picoplankton (cyanobacteria: *Anacystis nidulans, Synechococcus cedrorum, Synechococcus* 6911, *Synechococcus* PCC6301, and the rhodophyte *Cyanidium caldarium*). Other larger algal taxa that produce these VOCs are also given (source numbers as in Table 15-1).

Compound (reference)	Odor	Picoplankton	Other Taxa
Dihydrogen sulfide (18)	Rotten eggs	*Anacystis nidulans, Synechococcus cedrorum*	*Anabaena cylindrica, Oedogonium* sp., *Oscillatoria tenuis,*[II] *Oscillatoria chalybea,*[Φ] *Phormidium autumnale, Plectonema boryanum*
Dimethylsulfide (18)	Intense, sulfurous	*Anacystis nidulans, Synechococcus cedrorum*	*Anabaena cylindrica, Oscillatoria chalybea,*[Φ] *Oscillatoria tenuis*[II] *Phormidium autumnale, Plectonema boryanum, Nostoc*
Methyl mercaptan (18)		*Synechococcus cedrorum*	
2-Methylisoborneol, or MIB (31)	Sulfurous	*Synechococcus* sp. (not all strains)	See Tables 15-1, 15-3
β-Ionone (8)	Violets	*Synechococcus* 6911, *Cyanidium caldarium*	*Anabaena* sp., *A. cylindrica, Aphanizomenon gracile, Scenedesmus subspicatus, Synura uvella*
3-Methylbut-2-en-1-ol (22)		*Synechococcus* 6301	*Anabaena cylindrica*
6-Methyl-5-hepten-2-ol (5, 8, 9, 20)	Fruity; esterlike	*Synechococcus* 6911	
6-Methyl-5-hepten-2-one (5, 8, 9, 20)	Fruity; esterlike	*Cyanidium caldarium, Synechococcus* 6311, *S.* 6301	*Anabaena cylindrica, Aulacosiera granulata, Microcystis aeruginosa, Scenedesmus subspicatus, Synura uvella*
Methyl propionate (4)		*Synechococcus* 6911	*Ochromonas danica, Synura uvella*
Epoxy-β-ionone (5, 8, 9, 14)		*Synechococcus* 6911	
Dihydroactinidiolide (25)	Pungent violet	*Synechococcus* 6311, *S.* 6301 *(= Anacystis nidulans)*	
Geraniol (6, 25)	Sweet, floral, fruity	*Synechococcus* 6911	
Tetrahydrogeranyl-acetone (25)		*Synechococcus* 6911	
Nerol (25)	Sweet, rose	*Synechococcus* 6911	
Hexan-1-ol (5, 6)		*Synechococcus* 6911	*Scenedesmus subspicatus*
Heptan-1-ol (6)		*Synechococcus* 6911	
Octan-1-ol (6, 8, 10)	Orange-rose	*Synechococcus* 6911	
Oct-1-ene (8)		*Synechococcus* 6911	*Anabaena oscillarioides, Synura uvella*

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other microbial heterotrophs, may contribute to the benthic production of these two terpenoids (and other VOCs) (1) directly, sometimes concurrent with cyanobacterial communities; and (2) via the degradation of algal cells and release of cell-bound VOCs such as geosmin and MIB (Sugiura et al. 1994, Sugiura and Nakano 2000); and (3) through the general degradation of all organic material. These processes can produce significant spatial variation in VOC levels and high concentrations in lower water layers in some eutrophic lakes (Durrer et al. 1999), although in other lakes, little or no benthic production is observed (Zaitlin et al. 2003).

Similarly, zooplankton and other invertebrate grazers may release cell-bound compounds such as geosmin or trigger synthesis of induced VOCs such as oxylipins and some nor-carotenoids (above) through grazing activity. Zooplankton may themselves also be a significant source of VOCs (e.g., heptadecene; Wendel and Jüttner 1997).

Aquatic macrophytes also represent sources of odor. They provide organically rich surfaces that can support significant odor-producing biofilms (Watson and Ridal 2004) and contribute a variety of secondary metabolites such as phenols and other compounds (e.g., Gross 2003).

LARGE-SCALE FACTORS

Many processes are common to all waters, but it is important to recognize that each water body is unique and to develop an understanding of individual systems to diagnose and control odor events effectively. Age, size, shape, and location (latitude/altitude), local climate and catchment basin, agricultural/industrial/hydro/urban development, and water level drawdown/regulation shape the physics, chemistry, and biology of the water body and its resilience to human disturbance. They also influence both large-scale spatial and seasonal patterns in T&O, and many of the microscale processes that produce this odor. As shown in the preceding sections, biologically produced T&O may have one or more spatial origins in a given water body (Figure 15-5). VOCs can be derived from surface plankton, and less visible, deep-living, shoreline and terrestrial biota (Figure 15-3), and this can produce distinct or mixed seasonal and spatial patterns, especially in source waters with large and/or complex, highly subdivided basins or hydrological regimes (Watson et al. 2007a). Deep-living, benthic, and periphytic* ("hidden") T&O sources can be difficult to locate.

VOC diffusion and transfer by water movement and/or active/passive movement of algal cells can produce spatial and temporal lags between sites of VOC production and their detection in surface waters, and at intakes, beaches, and shorelines. These are especially important considerations for rivers, shallow lakes, and reservoirs, where more persistent VOCs produced at remote sources can be transported considerable distances to intakes (e.g., Izaguirre and Taylor 1995, Romero and Ventura 2000). Physical processes may also trigger abrupt and sometimes dramatic changes in T&O levels in raw water drawn from vertically stratified reservoirs and lakes, where segregated layers of planktonic or benthic VOC production remain undetected until physical processes such as mixing, drawdown, or (in large lakes such as the Great Lakes) downwelling transports them to the level of the intake pipes (Rao et al. 2003).

* That is, growing on submerged surfaces such as rocks, aquatic plants, and other biota.

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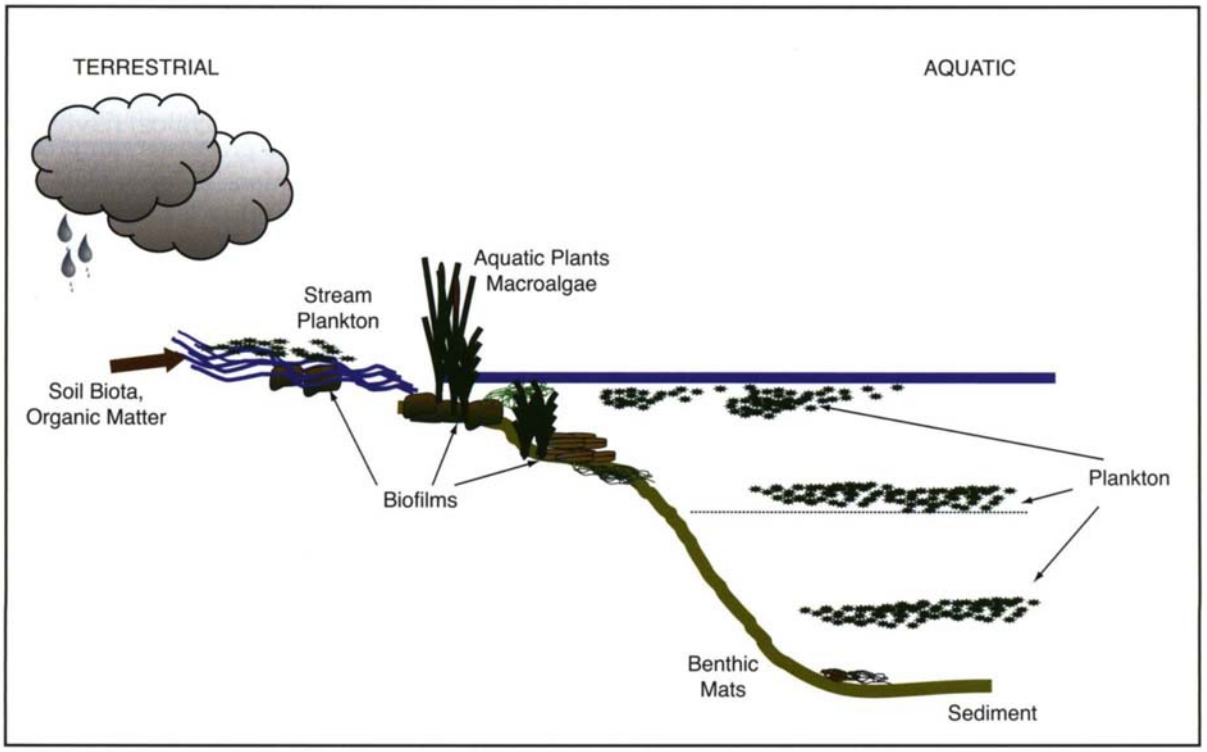

Figure 15-5 Terrestrial and aquatic biological sources of taste and odor

PROTOCOLS, MEASURES, AND OTHER CONSIDERATIONS

Sampling and Analyses

Sample design and the protocols used to collect and store/ship samples prior to analyses are critical to the interpretation of analytical results and diagnosing events. Inappropriate methods can render costly lab analyses and data interpretation effectively worthless. In particular:

1. It is important to investigate odor events using a sampling strategy that is based on a relevant frequency and spatial coverage.

2. Storage of unprocessed raw samples can result in changes in VOC concentrations though lysis and degradation of algal cells, headspace loss, photooxidation, or chemical/biological activity. This can be a major problem where there is a significant delay between sample collection and analyses (e.g., due to limited lab instrument accessibility or during extended field operations or shipping) and has motivated the development of innovative methods to stabilize samples for later recovery of important VOCs such as geosmin and MIB (e.g., Brownlee et al. 2004).

3. Some VOCs identified in samples may be contaminants (from solvents, extraction matrices, columns, etc.) or pollutants (e.g., halogenated and some aromatic compounds, and alkanes, e.g., undecane, nonane, decane, benzothiozole; Jüttner 1983, 1984) and need to be distinguished from those of biological origin. Chemicals (e.g., algicides, disinfectants, coagulants) or other treatments (e.g., ozone) applied in bloom control or water treatment may intensify T&O in the finished water by lysing cells or reacting with biological material to produce disinfection by-products (Peterson et al. 1995, Hargesheimer and Watson

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1996, Satchwill et al. 2007). It is of note here that algicides may also, in some cases, be ineffective against certain cyanobacteria species; for example, Izaguirre (1992) reported copper tolerance for a strong geosmin- and MIB-producing *Phormidium* species. Biological activity associated with filters and within the treatment plants may also produce potent VOCs (e.g., FAs, geosmin, 2,4,6-trichloroanisole; Karlsson et al. 1995, Jüttner and Watson 2007).

Abundance Measures: Critical Cell Densities

Similar to OTCs, critical cell densities (CDCs) are sometimes used to gauge the potential impact that a species may have on aquatic odor (or toxin) levels. Early studies based these values on empirical observations of abundance vs. detectable sample odor (e.g., Mallevialle and Suffet 1987; Table 15-4). More recently CDCs have been based on estimated per capita VOC production relative to OTCs (Table 15-5). These estimates may be misleading for several reasons. Most are based on measures of *in vitro* VOC yield at standard (and usually optimal) conditions—but as reviewed above, this is influenced by both endogenous and exogenous factors. Most species produce more than one odorous component, which when combined can modify overall odor levels. The relative proportions of major VOCs produced by a species vary over time and with environment/cell cycle. Mixed species field samples and sample matrices modify overall odor levels. Therefore, CDCs should be interpreted as general guidelines to be used for a preliminary assessment of water samples.

SUMMARY AND CONCLUSIONS

Algal and cyanobacterial T&O is a highly complex phenomenon and the above discussion is far from complete. Some key points are summarized:

- A diversity of VOCs are produced by a diversity of algal species; however, most T&O is caused by a relatively small number of these chemicals and taxa—and the majority of algal species have not been characterized.
- No single algal group or species has a unique "organoleptic fingerprint"; algal VOCs are produced by several or many species belonging to the same or different groups.
- Algae contribute to T&O: (1) Directly, by VOC production: (a) during active growth, which are released and/or stored (terpenoids, e.g., geosmin and MIB, nor-carotenoids, thiols); (b) *at cell damage and/or death* (oxylipins, e.g., fishy/cucumber-smelling PUAs, nor-carotenoids, and (2) Indirectly, with the release of VOCs during the degradation of their biomass.
- VOC production capacity and chemistry vary within and among species; co-occurring species may produce similar or different VOCs.
- Cell-bound VOC pools (e.g., geosmin) are often significant but incompletely measured by common analytical methods—which can also introduce contaminants or artifacts.
- VOC properties influence their persistence and fate in water and determine OTCs.
- T&O is not necessarily caused by the most abundant VOC or algal species.
- Cyanobacteria are major producers of VOCs *over growth*, notably geosmin and MIB; however, not all cyanobacteria produce these compounds or strong odors.
- Chrysophytes, synurophytes, and diatoms have a high PUFA cell content, which produces fishy/oily/floral/cucumber-smelling derivative (oxylipins) *at cell rupture.*

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Table 15-5 Critical cell densities estimated for specific VOCs (from measured cell production and OTCs) or from nonspecific sensory detection (SD)*

Taxa	Group	VOC	OTC (μg/L)	CDC Cells (mL^{-1})	Reference
Anabaena sp.	CYAN	SD		5.E+03	30
Anabaena laxa	CYAN	geosmin	0.004	2.E+02	7
Anabaena sp.	CYAN	β-cyclocitral	19.3	1.E+06	13
Anabaena sp.	CYAN	geosmin	0.004	4.E+01	28
Aphanizomenon	CYAN	SD		7.E+03	30
Microcystis sp.	CYAN	SD		4.E+04	30
Microcystis aeruginosa	CYAN	β-cyclocitral	19.3	2.E+05 -1.E+06	28, 13
Oscillatoria sp.	CYAN	SD		5.E+04	30
Oscillatoria cf. *chalybea*†	CYAN	MIB	0.029	1.E+02	7
Oscillatoria tenuis‡	CYAN	geosmin	0.004	1.E+03	7
Phormidium cf. *calcicola*	CYAN	geosmin	0.004	3.E+02	7
Phormidium cf. *calcicola*	CYAN	MIB	0.029	5.E+02	7
Dinobryon cylindricum	CHRY	2,4,7-decatrienal	1.5	4.E+03	29
D. cylindricum	CHRY	2,4-heptadienal	3	5.E+04	29
D. divergens	CHRY	2,4,7-decatrienal	1.5	8.E+03	29
Dinobryon sp.	CHRY	SD		3.E+03	30
Dinobryon sp.	CHRY	2,4-heptadienal	3	4.E+05	7
Mallomonas sp.	CHRY	SD		5.E+02	30
Synura sp.	CHRY	SD		3.E+02	30
Synura petersenii	CHRY	2,6 -nonadienal	0.08	3.E+02	7
Synura petersenii	CHRY	2,4,7-decatrienal	1.5	2.E+03	7
Uroglena americana	CHRY	2,4,7-decatrienal	1.5	1.E+05	29
Uroglena americana	CHRY	2,4-heptadienal	3	3.E+04	29
Ceratium sp.	DIN	SD		2.E+02	30
Cryptomonas sp.	CRYP	SD		1.E+03	30
Asterionella formosa	DIAT	2,4,7-octatriene	No data	3.E+03	30
Cyclotella sp.	DIAT	SD		2.E+03	30
Melosira (Aulacoseira) sp.	DIAT	SD		3.E+03	30
Synedra sp.	DIAT	SD		3.E+03	30
Tabellaria sp.	DIAT	SD		8.E+02	30
Euglena sp.	CHLOR	SD		8.E+02	30
Ankistrodesmus sp.	CHLOR	SD		4.E+03	30
Chlamydomonas sp.	CHLOR	SD		4.E+03	30
Eudorina sp. (colonies)	CHLOR	SD		8.E+01	30
Pandorina sp.	CHLOR	SD		2.E+03	30
Scenedesmus sp. (colonies)	CHLOR	SD		2.E+03	30

*Taxonomic group abbreviations as in Table 15-1. References as in Table 15-2. Synonyms for revised species names given in table.
†*Phormidium chalybeum.*
‡*Phormidium tenue.*

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- Chlorophytes are most commonly associated with green leaf alcohols.
- Other algal taxonomic and size groups have been less well characterized for their contributions to T&O; picoplankton may represent an overlooked odor source in some waters.
- T&O can occur without visible surface blooms; VOCs can be derived from surface plankton and "hidden," deep-living, shoreline, and terrestrial biota.
- VOC diffusion and transfer by water movement and/or active/passive movement of cells can produce spatial and temporal lags between sites of VOC production and detection.
- T&O and toxin production are poorly correlated.

Consumers perceive the T&O of drinking water as a measure of its safety. Toxic cyanobacterial blooms are now a very real concern across the water industry, governments, public, and other stakeholders, with an increasing need to identify and assess the risk(s) involved. Although T&O is itself not a health risk, it may be associated with, or produced, by potentially toxic taxa, notably certain cyanobacteria, and is often used as a surrogate olfactory signal of the presence of toxic taxa, although this link is tenuous and inconsistent. To both managers and aquatic ecologists, T&O can provide clues to some of the mechanisms that produce changes in the structure and dynamics of the biological community. From this perspective, T&O events represent opportunities to increase our understanding of chemical interactions among organisms, and how these may lead to or reflect changes at the level of the individual and population.

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REFERENCES

Anagnostidis, K., and J. Komárek. 1988. Modern approach to the classification of cyanophytes 3. Oscillatoriales. *Arch. Hydrobiol./ Suppl. 80. Algol. Stud.*, 50–53:327–472.

Andersen, R. 1987. Synurophyceae classis nov., a new class of algae. *Am. Jour. Bot.*, 74:337–353.

Anthoni, U., C. Christophersen, C. Madsen, J. Ogaard, S. Wium-Andersen, and N. Jacobsen. 1980. Biologically active sulphur compounds from the green alga *Chara globularis*. *Phytochem.*, 19:1228–1229.

Bankova, V., K. Stefanov, S. Dimitrova-Konaklieva, G. Keremedchieva, X. Frette, C. Nikolova, A. Kujumgiev, and S. Popov. 2001. Secondary metabolites and lipids in *Chara globularis* Thuill. *Hydrobiologia*, 457:199–203.

Bao, M.-L., K. Barbieri, D. Burrini, O. Griffini, and F. Pantini. 1997. Determination of trace levels of taste and odor compounds in water by microextraction and gas-chromatography-ion-trap detection-mass spectrometry. *Water Res.*, 31:1719–1722.

Bartels, J., G. Burlingame, and I. Suffet. 1986. Flavor Profile Analysis: Taste and odor control of the future. *Jour. AWWA*, 78:50–55.

Bechard, M., and W. Rayburn. 1979. Volatile organic sulphides from freshwater algae. *Jour. Phycol.*, 15:379–383.

Benanou, D., F. Acobas, and M.R. de Roubin. 2004. Optimization of stir bar sorptive extraction applied to the determination of odorous compounds in drinking water. *Water Sci. Technol.*, 49:161–170.

Berger, J., and M. Schagerl. 2004. Allelopathic activity of Characeae. *Biologia, Bratislava*, 59:9–15.

Berglind, L., H. Holtan, and O. Skulberg. 1983. Case studies on off-flavors in some Norwegian lakes. *Water Sci. Technol.*, 15:199–209.

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Blevins, W.T., K.K. Schrader, and I. Saadoun. 1995. Comparative physiology of geosmin production by *Streptomyces halstedii* and *Anabaena* sp. *Water Sci. Technol.*, 31:127–133.

Bowmer, K., A. Padovan, R. Oliver, W. Korth, and G. Ganf. 1992. Physiology of geosmin production by *Anabaena circinalis* isolated from the Murrumbidgee River, Australia. *Water Sci. Technol.*, 25:259–267.

Brownlee, B.G., D.S. Painter, and R.J. Boone. 1984. Identification of taste and odor compounds from western Lake Ontario. *Water Pollut. Res. Jour. Can.*, 19:111–118.

Brownlee, B., G. MacInnis, M. Charlton, S. Watson, S. Hamilton-Browne, and J. Milne. 2004. An analytical method for shipboard extraction of the odor compounds, 2-methylisoborneol and geosmin. *Water Sci. Technol.*, 49:121–127.

Brownlee, B., C. Marvin, G. MacInnis, M. Charlton, and S. Watson. 2007. Interlaboratory comparison of geosmin and 2-methylisoborneol in municipal tap water. *Water Sci. Technol.*, 55(5):51–57.

Bruchet, A. 1999. Solved and unsolved cases of taste and odor episodes in the files of Inspector Cluzeau. *Water Sci. Technol.*, 40:15–21.

Bruchet, A., and J. Laîné. 2005. Efficiency of membrane processes for taste and odor removal. *Water Sci. Technol.*, 51:257–265.

Burlingame, G., J. Muldowney, and R. Maddrey. 1992. Cucumber flavor in Philadelphia's drinking water. *Jour. AWWA*, 91:92–97.

Carail, M., and C. Caris-Veyrat. 2006. Carotenoid oxidation products: From villain to savior? *Pure Appl. Chem.*, 78:1493–1503.

Carrick, H.J., and C. Schelske 1997. Have we overlooked the importance of small phytoplankton in productive waters? *Limnol. Oceanogr.*, 42:1613–1621.

Chiang, I., W. Huang, and J. Wu. 2004. Allelochemicals of *Botryococcus braunii* (Chlorophyceae). *Jour. Phycol.*, 40:474–480.

Chorus, I., G. Klein, and W. Rotard. 1990. Volatile substances associated with algal blooms of different trophic state and in bank filtrate. *Verh. Int. Verein. Limnol.*, 24:270–273.

Chrost, R.J., and A.J. Gajewski. 1995. Microbial utilization of lipids in lakewater. *FEMS Microb. Ecol.*, 18:45–50.

Chung, S., J. Vercellotti, P. Johnsen, and P. Klesius. 1991. Development of an ELISA for geosmin. *Jour. Agric. Food Chem.*, 39:764–769.

Collins, R., and K. Kalnins. 1965. Volatile constituents produced by the alga *Synura petersenii*. II: Alcohols, esters and acids. *Int. Jour. Air Water Pollut.*, 9:501–504.

Cook, D., and G. Newcombe. 2004. Can we predict the removal of MIB and geosmin with PAC by using water quality parameters? *Water Sci. Technol. Water Supply*, 4:221–226.

Cotsaris, E., A. Bruchet, J. Mallevialle, and D. Bursill. 1995. The identification of odorous metabolites produced from algal monocultures. *Water Sci. Technol.*, 31:251–258.

Davies, J.M., M. Roxborough, and A. Mazumder. 2004. Origins and implications of drinking water odors in lakes and reservoirs of British Columbia, Canada. *Water Res.*, 38:1900–1910.

Dembitsky, V.M., I. Shkrob, and O. Lev. 2000. Occurrence of volatile nitrogen-containing compounds in nitrogen-fixing cyanobacterium *Aphanizomenon flos-aquae*. *Jour. Chem. Ecol.*, 26:1359–1366.

Dietrich, A., M. Orr, D. Gallagher, and R. Hoehn. 1992. Tastes and odors associated with chlorine dioxide. *Jour. AWWA*, 84:82–88.

Dietrich, A.M., A.J. Whelton, R.C. Hoehn, R. Anderson, and M. Wille. 2004. The attribute rating test for sensory analysis. *Water Sci. Technol.*, 49:61–67.

Durrer, M., U. Zimmermann, and F. Jüttner. 1999. Dissolved and particle-bound geosmin in a mesotrophic lake (Lake Zurich): Spatial and seasonal distribution and the effect of grazers. *Water Res.*, 33:3628–3636.

Eriksson, C.E., B. Lundgren, and K. Vallentin. 1976. Odor detectability of aldehydes and alcohols originating from lipid oxidation. *Chem. Senses*, 2:3–15.

Fink, P., E. von Elert, and F. Jüttner. 2006. Volatile foraging kairomones in the littoral zone: Attraction of an herbivorous freshwater gastropod to algal odors. *Jour. Chem. Ecol.*, 32:1867–1831.

Fontana, A., G. d'Ippolito, A. Cutignano, A. Miralto, A. Ianora, G. Romano, and G. Cimino. 2007. Chemistry of oxylipin pathways in marine diatoms. *Pure Appl. Chem.*, 79:481–490.

Gardiner, H. 1991. Recent investigations into the lipoxygenases pathways of plants. *Biochem. Biophys. Acta*, 1084:221–239.

Gross, E. 2003. Allelopathy of aquatic autotrophs. *Crit. Rev. Plant Sci.*, 22:313–339.

Hargesheimer, E., and S. Watson. 1996. Drinking water treatment options for taste and odor control. *Water Res.*, 6:1423–1430.

Hayes, M., and M. Burch. 1989. Odorous compounds associated with algal blooms

Copyright (C) 2010 American Water Works Association All Rights Reserved

in South Australian waters. *Water Res.*, 23:115–123.

Henatsch, J., and F. Jüttner. 1983. Volatile odorous excretion products of different strains of *Synechococcus* (Cyanobacteria). *Water Sci. Technol.*, 15:259–266.

Herrmann, V., and F. Jüttner. 1977. Excretion products of algae. Identification of biogenesis amines by gas–liquid chromatography and mass spectrometry of their trifluoroacetamides. *Anal. Biochem.*, 78:365–373.

Höckelmann, C., and F. Jüttner. 2004. Volatile organic compound (VOC) analysis and sources of limonene, cyclohexanone and straight chain aldehydes in axenic cultures of *Calothrix* and *Plectonema*. *Water Sci. Technol.*, 49:47–54.

Höckelmann, C., T. Moens, and F. Jüttner. 2004. Odor compounds from cyanobacterial biofilms acting as attractants and repellents for free-living nematodes. *Limnol. Oceanogr.*, 49:1809–1819.

Hofbauer, B., and F. Jüttner. 1988. Occurrence of isopropylthio compounds in the aquatic ecosystem Lake Neusiedl Austria as a chemical marker for *Microcystis flos-aquae*. *FEMS Microb. Ecol.*, 53:113–122.

Hrudey, S.E., D. Rector, and N. Motkosky. 1992. Characterization of drinking water odor arising from spring thaw for an ice-covered upland river source. *Water Sci. Technol.*, 25:65–72.

Izaguirre, G. 1992. A copper-tolerant *Phormidium* species from Lake Mathews, California, that produces 2-methylisoborneol and geosmin. *Water Sci. Technol.*, 31(11):41–48.

Izaguirre, G. 2007. Geosmin- and MIB-producing cyanobacteria found in the United States. In *Early warning and management of surface water taste-and-odor events.* W.D. Taylor, R.F. Losee, M. Torobin, G. Izaguirre, D. Sass, D. Khiari, and K. Atasi, eds. Denver, Colo.: AWWA.

Izaguirre, G., and W.D. Taylor. 1995. Geosmin and 2-methylisoborneol production in a major aqueduct system. *Water Sci. Technol.*, 31:41–48.

Izaguirre, G., C. Hwang, S. Krasner, and M. McGuire. 1982. Geosmin and 2-methylisoborneol from cyanobacteria in three water supply systems. *Appl. Environ. Microbiol.*, 43:708.

Izaguirre, G., C. Hwang, S. Krasner, and M. McGuire. 1983. Production of 2-methylisoborneol by two benthic cyanobacteria. *Water Sci. Technol.* 15(6/7):211–220.

Izaguirre, G., W.D. Taylor, and J. Pasek. 1999. Off-flavor problems in two reservoirs associated with planktonic *Pseudanabaena* species. *Water Sci. Technol.*, 40(6):85–90.

Jeannot, M., and F. Cantwell. 1996. Solvent microextraction into a single drop. *Anal. Chem.*, 68:2236–2240.

Jensen, S., C. Anders, L. Goatcher, T. Perley, S. Kenefick, and S. Hrudey. 1994. Actinomycetes as a factor in odor problems affecting drinking water from the North Saskatchewan River. *Water Res.*, 28:1393–1401.

Jiang, J., X. He, and D.E. Cane. 2006. Geosmin biosynthesis. *Streptomyces coelicolor* germacradienol/germacrene D synthase converts farnesyl diphosphate to geosmin. *Jour. Am. Chem. Soc.*, 128:8128–8129.

Jones, G., and W. Korth. 1995. *In situ* production of volatile odor compounds by river and reservoir phytoplankton populations in Australia. *Water Sci. Technol.*, 31(11):145–151.

Jüttner, F. 1979. Nor-carotenoids as the major volatile excretion products of *Cyanidium*. *Z. Naturforsch.*, 34c:186–191.

Jüttner, F. 1981a. Biologically active compounds released during algal blooms. *Verh. Int. Verein. Limnol.*, 21:227–230.

Jüttner, F. 1981b. Detection of lipid degradation products in the water of a reservoir during a bloom of *Synura uvella*. *Appl. Environ. Microbiol.*, 41:100–106.

Jüttner, F. 1983. Volatile odourous excretion products of algae and their occurrence in the natural aquatic environment. *Water Sci. Technol.*, 15:247–257.

Jüttner, F. 1984. Dynamics of the volatile organic substances associated with cyanobacteria and algae in a eutrophic shallow lake. *Appl. Environ. Microbiol.*, 47:814–820.

Jüttner, F. 1992. Flavor compounds in weakly polluted rivers as a means to differentiate pollution sources. *Water Sci. Technol.*, 25(2):155–164.

Jüttner, F. 1995. Physiology and biochemistry of odourous compounds from freshwater cyanobacteria and algae. *Water Sci. Technol.*, 31:69–78.

Jüttner, F. 2001. Liberation of 5,8,11,14,17-eicosapentaenoic acid and other polyunsaturated fatty acids from lipids as a grazer defense reaction in epilithic diatom biofilms. *Jour. Phycol.*, 37:744755.

Jüttner, F., and U. Dürst. 1997. High lipoxygenase activities in biofilms of diatoms. *Arch. Hydrobiol.*, 138:451–463.

Jüttner, F., and B. Hahne. 1981. Volatile excretion products of *Poteriochromonas malhamensis*. Identification and formation. *Z. Pflanzenphys.*, 103:403–412.

Copyright (C) 2010 American Water Works Association All Rights Reserved

Jüttner, F., and R. Hans. 1986. The reducing capacities of cyanobacteria for aldehydes and ketones. *Appl. Microbiol. Biotechnol.*, 25:52–54.

Jüttner, F., and B. Höflacher. 1985. Evidence of ß-carotene 7,8(7',8') oxygenase (ß-cyclocitral, crocetindial generating) in *Microcystis Arch. Microbiol.*, 141:337–343.

Jüttner, F., and H. Müller. 1979. Excretion of octadiene and octatrienes by a freshwater diatom. *Naturwiss*, 66:363–364.

Jüttner, F., and S.B. Watson. 2007. Biochemical and ecological control of geosmin and 2-methylisoborneol in source waters. *Appl. Environ. Microbiol.,* 73(14):4395–4406.

Jüttner, F., E. Wiedemann, and K. Wurster. 1982. Excretion of S- and O-methyl esters and other volatile compounds by *Ochromonas danica. Phytochemistry*, 21:2185–2188.

Jüttner, F., and K. Wurster. 1984. Evidence of ectocarpene and dictyopterenes A and C' in the water of a freshwater lake. *Limnol. Oceanogr.*, 29:1322–1324.

Jüttner, F., B. Hoflacher, and K. Wurster. 1986. Seasonal analysis of volatile organic biogenic substances (VOBS) in freshwater phytoplankton populations dominated by *Dinobryon, Microcystis* and *Aphanizomenon. Jour. Phycol.*, 22:169–175.

Kamiya, H., K. Naka, and K. Hashamito. 1978. Ichthyotoxicity of a flagellate *Uroglena volvox. Bull. Jpn. Soc. Sci. Fish.,* 45:129.

Karahadian, C., and R. Lindsay. 1989. Evaluation of compounds contributing characterizing fishy flavors in oils. *Jour. Am. Oil Chem. Soc.*, 66:953–959.

Karlsson, S., A. Grimvall, and H. Borén. 1995. Drinking water off-flavor caused by the formation of short-chain fatty acids in slow sand filters. *Water Sci. Technol.*, 31:49–54.

Kenefick, S., S. Hrudley, E. Prepas, N. Motkosky, and H. Peterson. 1992. Odorous substances and cyanobacterial toxins in prairie drinking water sources. *Water Sci. Technol.*, 25(2):147–154.

Khiari, D., I. Suffet, and S.E. Barrett. 1995. The determination of compounds causing fishy/swampy odors in drinking water supplies. *Water Sci. Technol.*, 31:105–112.

Klausen, C., M. Nicolaisen, B. Strobel, F. Warnecke, J. Nielsen, and N. Jørgensen. 2004. Abundance of actinobacteria and production of geosmin and 2-methylisoborneol in Danish streams and fish ponds. *FEMS Microbiol. Ecol.*, 52:265–278.

Krasner, S., M. McGuire, and V. Ferguson. 1985. Tastes and odors: The flavor profile method. *Jour. AWWA*, 77:34–39.

Liersch, R. 1976. Über das ätherische Öl von Grünalgen. 1. Die Öle der Gattung *Chlorella. Arch. Microbiol.*, 107:353–356.

Lin, S.D., R.L. Evans, and D.B. Beuscher. 1979. Phytoplankton in the Fox chain of lakes. *Jour. AWWA*, 15:974–983.

Lynn, S.G., S.S. Kilham, D.A. Kreeger, and S.J. Interlandi. 2000. Effect of nutrient availability on the biochemical and elemental stoichiometry in the freshwater diatom *Stephanodiscus minutulus* (Bacillariophyceae). *Jour. Phycol.*, 36:510–522.

Mallevialle, J., and I. Suffet, eds. 1987. Identification and treatment of tastes and odors in drinking water. Denver, Colo.: AWWA.

Matsumoto, A., and Y. Tsuchiya. 1988. Earthy-musty odor-producing cyanophytes isolated from five water areas in Tokyo. *Water Sci. Technol.*, 20:179–183.

McBean, E.A. 1990. Key factors in taste and odor problems for municipal water works using surface water sources. *Ergebin. Limnol.*, 33:888–891.

Meijboom, P., and J. Stroink. 1972. 2-*trans*, 4-*cis*, 7-*cis*-decatrienal, the fishy off-flavor occurring in strongly autoxidized oils containing linolenic acid or ω3,6,9, etc., fatty acids. *Jour. Am. Oil Chem. Soc.*, 49:555–558.

Means E.G., II, and M. McGuire. 1986. Early warning system for taste and odor control. *Jour. AWWA*, 78:77–83.

Mohren, S., and F. Jüttner. 1983. Odorous compounds of different strains of *Anabaena* and *Nostoc* (cyanobacteria). *Water Sci. Technol.*, 15:221–223.

Naes, H., H. Utkilen, and A. Post. 1988. Factors influencing geosmin production by the cyanobacterium *Oscillatoria brevis. Water Sci. Technol.*, 20:125–131.

Napolitano, G. 1994. The relationship of lipids with light and chlorophyll measurements in freshwater algae and periphyton. *Jour. Phycol.*, 30:943–950.

Nicholls, K.H. 1982. Lakewide odors in Ontario and New Hampshire caused by *Chrysochromulina breviturrita* (Prymnesiophyceae). *Hydrobiologia*, 96:91–95.

Nicholls, K.H. 1995. Chrysophyte blooms in the plankton and neuston of marine and freshwater systems. In *Chrysophyte algae.* C. Sandgren, J. Smol, and J. Kristiansen, eds. Cambridge, UK: Cambridge University Press.

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Oestman, E., L. Schweitzer, P. Tomboulian, A. Corado, and I.H.A. Suffet. 2004. Effects of chlorine and chloramines on earthy and musty odors in drinking water. *Water Sci. Technol.*, 49:153–159.

Oikawa, E., and Y. Ishibashi. 2004. Species specificity of musty odor producing *Phormidium tenue* in Lake Kamafusa. *Water Sci. Technol.*, 49:41–46.

Park, G., M. Yu, J.-Y. Koo, W.H. Joe, and H. Kim. 2006. Oxidation of geosmin and MIB in water using O_3/H_2O_2: Kinetic evaluation. *Water Sci. Technol: Water Supply*, 6:63–69.

Persson, P.-E. 1982. Muddy odor: A problem associated with extreme eutrophication. *Hydrobiologia*, 86:1–2.

Persson, P.-E. 1983. Off-flavors in aquatic ecosytems—An introduction. *Water Sci. Technol.*, 15:1–11.

Persson, P.-E. 1988. Odorous algal cultures in culture collections. *Water Sci. Technol.*, 20:211–213.

Persson, P.-E. 1996. Cyanobacteria and off-flavors. *Phycologia*, 35:168–171.

Persson, P.-E., and F. Jüttner. 1983. Threshold odor concentrations of odorous algal metabolites occurring in lake water. *Aqua Fenn.*, 13:3–7.

Persson, P.-E., and K. Sivonen. 1979. Notes on muddy odor. V. Actinomycetes as contributors to muddy odor in water. *Aqua Fenn.*, 9:57–61.

Peterson, H.G., S.E. Hrudey, I.A. Cantin, T.R. Perley, and S.L. Kenefick. 1995. Physiological toxicity, cell membrane damage and the release of dissolved organic carbon and geosmin by *Aphanizomenon flos-aquae* after exposure to water treatment chemicals. *Water Res.*, 29:1515–1523.

Pohnert, G., and E. von Elert. 2000. No ecological relevance of trimethylamine in fish—*Daphnia* interactions. *Limnol. Oceanogr.*, 45:1153–1156.

Polak, E.H., and J. Provasi. 1992. Odor sensitivity to geosmin enantiomers. *Chem. Senses*, 17:23–26.

Preti, G., T.S. Gittelman, P.B. Staudte, and P. Luitweiler. 1993. Letting the nose lead the way: Malodorous components in drinking water. *Anal. Chem.*, 65:699–702.

Rao, Y.R., M.G. Skafel, T. Howell, and R.C. Murthy. 2003. Physical processes controlling taste and odor episodes in Lake Ontario drinking water. *Jour. Great Lakes Res.*, 29:70–78.

Rashash, D., A. Dietrich, R. Hoehn, and B. Parker. 1995. The influence of growth conditions on odor-compound production by two chrysophytes and two Cyanobacteria. *Water Sci. Technol.*, 31(11):165–172.

Rashash, D., R. Hoehn, A. Dietrich, and T. Grizzard. 1996. Identification and control of odorous algal metabolites. Denver, Colo.: AWWA.

Rechardt, W. 1981. Influence of methylheptenone and related phytoplankton nor-carotenoids on heterotrophic aquatic bacteria. *Can. Jour. Microbiol.*, 27:144–147.

Richards, S.R., C. Kelly, and J. Rudd. 1991. Organic volatile sulfur in lakes of the Canadian Shield and its loss to the atmosphere. *Limnol. Oceanogr.*, 36:468–482.

Ridal, J.J., S.B. Watson, and M. Hickey. 2007. A comparison of biofilms from macrophytes and rocks for taste and odor producers in the St. Lawrence River. *Water Sci. Technol.*, 55:15–21.

Romero, J., and F. Ventura. 2000. Occurrence of geosmin and other odorous compounds of natural origin in surface and drinking waters. A case study. *Int. Jour. Environ. Anal. Chem.*, 77:243–254.

Rosen, B., B. MacLeod, and M. Simpson. 1992. Accumulation and release of geosmin during the growth phases of *Anabaena circinalis* (Kutz.) Rabenhorst. *Water Sci. Technol.*, 25:185–190.

Rzama, A., A. Benharref, B. Arreguy, and E. Dufourc. 1995. Volatile compounds of green microalgae grown on reused waste water. *Phytochemistry*, 38:1375–1379.

Saadoun, I., and M. El. 1998. Degradation of geosmin-like compounds by selected species of Gram-positive bacteria. *Lett. Appl. Microbiol.*, 26:98–100.

Saadoun, I., K. Schrader, and W. Blevins. 1997. Identification of 2-methylisoborneol (MIB) and geosmin as volatile metabolites of *Streptomyces violaceusniger*. *Actinomycetes*, 8:37–41.

Saito, A., T. Tokuyama, A. Tanaka, T. Oritani, and K. Fuchigami. 1999. Microbiological degradation of (–)-geosmin. *Water Res.*, 33:3033–3036.

Satchwill, T., S.B. Watson, and E. Dixon. 2007. Odorous algal-derived alkenes: Differences in stability and treatment responses in drinking water. *Water Sci. Technol.*, 55(5):95–102.

Schauenstein, E., H. Esterbauer, and H. Zollner. 1977. *Aldehydes in biological systems*. London: Pion.

Schrader, K.K., and W.T. Blevins. 1993. Geosmin-producing species of *Streptomyces* and *Lyngbya* from aquaculture ponds. *Can. Jour. Microbiol.*, 39:834–840.

Skjevrak, I., V. Lund, K. Ormerod, and H. Herikstad. 2005. Volatile organic compounds in natural biofilm in polyethylene pipes supplied with lake water and treated

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water from the distribution network. *Water Res.*, 39:4133–4141.

Soma, Y., T. Imaizumi, K. Yagi, and S. Kasuga. 1993. Estimation of algal succession in lake water using HPLC analysis of pigments. *Can. Jour. Fish. Aquat. Sci.*, 50:1142–1146.

Spiteller, D., A. Jux, J. Piel, and W. Boland. 2002. Feeding of (5,5-2H2)-1-desoxy-D-xylulose and (4,4,6,6,6-2H5)-mevalolactone to a geosmin-producing *Streptomyces* sp. and *Fossombronia pusilla*. *Phytochemistry*, 61:827–834.

Stahl, P., and T. Parkin. 1994. Purge-and-trap extraction of geosmin and 2-methylisoborneol from soil. *Soil Sci. Soc. Am. Jour.*, 58:1163–1167.

Sugiura, N., and K. Nakano. 2000. Causative microorganisms for musty odor occurrence in the eutrophic Lake Kasumigaura. *Hydrobiologia*, 434:145–150.

Sugiura, N., Y. Inamori, Y. Hosaka, R. Sudo, and G. Takahashi. 1994. Algae enhancing musty odor production by actinomycetes in Lake Kasumigaura. *Hydrobiologia*, 288:57–64.

Sugiura, N., N. Iwami, Y. Inamori, O. Nishimura, and R. Sudo. 1998. Significance of attached cyanobacteria relevant to the occurrence of musty odor in Lake Kasumigaura. *Water Res.*, 32:3549–3554.

Sumitomo, H. 1992. Biodegradation of 2-methylisoborneol by gravel sand filtration. *Water Sci. Technol.*, 25:191–198.

Tabachek, J.A., and M. Yurkowski. 1976. Isolation and identification of blue-green algae producing muddy odor metabolites, geosmin, and 2-methylisoborneol, in saline lakes in Manitoba. *Jour. Fish. Res. Board Can.*, 33:25–35.

Tanaka, A., T. Oritani, F. Uehara, A. Saito, H. Kishita, Y. Niizeki, H. Yokota, and K. Fuchigami. 1996. Biodegradation of a musty odor component, 2-methylisoborneol. *Water Res.*, 30:759–761.

Theimer, E., T. Yoshida, and E. Klaiber. 1977. Olfaction and molecular shape: Chirality as a requisite for odor. *Jour. Agric. Food Chem.*, 25:1168–1177.

Tsuchiya, Y., and A. Matsumoto. 1988. Identification of volatile metabolites produced by blue-green algae. *Water Sci. Technol.*, 20:149–155.

Tsuchiya, Y., K. Shudo, and T Okamoto. 1979. The odorous compounds in the blue-green algae, *Oscillatoria* sp., and the river water: Identification of 2-methylisoborneol, geosmin, pcresol, indol and 3-methylindole. *Eisei Kagaku*, 25: 216–220.

Tsuchiya, Y., M. Watanabe and M. Watanabe, 1992. Volatile organic sulfur compounds associated with blue-green algae from inland waters of Japan. *Wat. Sci. Technol.* 25(2): 123–130.

Tyler, L.D., T.E. Acree, and R.M. Butts. 1978. Odor characterization of the synthetic stereoisomers of 2-methylborneol. *Jour. Agric. Food Chem.*, 26:1415–1417.

Utkilen, H.C., and M. Frøshaug. 1992. Geosmin production and excretion in a planktonic and benthic *Oscillatoria*. *Water Sci. Technol.*, 25:199–206.

van der Ploeg, M., M.E. Dennis, and M.Q. de Regt. 1995. Biology of *Oscillatoria* cf *chalybea*, a 2-methylisoborneol producing blue-green alga of Mississippi catfish ponds. *Water Sci. Technol.*, 31:173–180.

Velzeboer, R., M. Drikas, C. Donati, M. Burch, and D. Steffensen. 1995. Release of geosmin by *Anabaena circinalis* following treatment with aluminium sulphate. *Water Sci. Technol.*, 31:187–194.

Vilalta, E., H. Guasch, I. Munoz, A. Romani, F. Valero, J.J. Rodriguez, R. Alcaraz, and S. Sabater. 2004. Nuisance odors produced by benthic cyanobacteria in a Mediterranean river. *Water Sci. Technol.*, 49:25–31.

Wake, L., and L. Hillen. 1981. Nature and hydrocarbon content of blooms of the alga *Botryococcus braunii* occurring in Australian freshwater lakes. *Aust. Jour. Mar. Freshwater Res.*, 32:353–367.

Watson, S.B. 1999. Outbreaks of taste/odour causing algal species: theoretical, mechanistic and applied approaches. Ph.D. Thesis, University of Calgary, Alta.

Watson, S.B. 2003. Chemical communication or chemical waste? A review of the chemical ecology of algal odor. *Phycologia*, 42:333–350.

Watson, S.B. 2004. Aquatic taste and odor: A primary signal of drinking water integrity. *Jour. Toxicol. Environ. Health A*, 67(20–22): 1779–1795.

Watson, S.B., and T. Satchwill. 2003. Chrysophyte odor production: The impact of resources at the cell and population levels. *Phycologia*, 42:393–405.

Watson, S.B., and J. Ridal. 2004. Periphyton: A primary source of widespread and severe taste and odor. *Water Sci. Technol.*, 49(9):33–39.

Watson, S.B., B. Brownlee, and T. Satchwill. 1998. The use of solid-phase microextraction (SPME) as a simple method for the detection and monitoring of off-flavour compounds in drinking water supplies. In *Proceedings, WCWWA 50th Annual Conference*, Calgary, Alta. pp. 451–457.

Watson, S.B., B. Brownlee, T. Satchwill, and E. McCauley. 1999a. The use of solid phase microextraction (SPME) to monitor for

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major organoleptic compounds produced by chrysophytes in surface waters. *Water Sci. Technol.*, 40:251–256.

Watson, S.B., T. Satchwill, and B. Brownlee. 1999b. Quantitative analysis of trace levels of geosmin and MIB in source and drinking-water using headspace SPME. *Water Res.*, 34:2818–2828.

Watson, S.B., T. Satchwill, and E. McCauley. 2001a. Drinking water taste and odor: A chrysophyte perspective. *Nova Hedwig*, 122:119–146.

Watson, S.B., T. Satchwill, and E. McCauley. 2001b. Under-ice blooms and source-water odor in a nutrient-poor reservoir: Biological, ecological and applied perspectives. *Freshwater Biol.*, 46:1–15.

Watson, S.B., J. Ridal, B. Zaitlin, and A. Lo. 2003. Odors from pulp mill effluent treatment ponds: The origin of significant levels of geosmin and 2-methylisoborneol (MIB). *Chemosphere*, 51:765–773.

Watson, S.B., M. Charlton, Y.R. Rao, T. Howell, J. Ridal, B. Brownlee, C. Marvin, and S. Millard. 2007a. Off flavors in large water bodies: Physics, chemistry and biology in synchrony. *Water Sci. Technol.*, 55(5):1–8.

Watson, S.B., F. Jüttner, and O. Köster. 2007b. *Daphnia* behavioral responses to taste and odor compounds: Ecological significance and application as an inline treatment plant monitoring tool. *Water Sci. Technol.*, 55(5):23–31.

Watson, S.B., J. Ridal, and G. Boyer. 2008. Taste and odor and cyanobacterial toxins: Impairment, prediction, and management in the Great Lakes. *Can. Jour. Fish. Aquat. Sci.*, 65(8):1779–1796.

Wee, J., S. Harris, J. Smith., C. Dionigi, and D. Millie. 1994. Production of the taste/odor compound, *trans*-2, *cis*-6-nonadienal within the Synurophyceae. *Jour. Appl. Phycol.*, 6:365–369.

Wendel, T., and F. Jüttner. 1996. Lipoxygenase-mediated formation of hydrocarbons and unsaturated aldehydes in freshwater diatoms. *Phytochemistry*, 41:1445–1449.

Wendel, T., and F. Jüttner. 1997. Excretion of heptadecene-1 into lake water by swarms of *Polyphemus pediculus* (Crustacea). *Freshwater Biol.*, 38:203–207.

Weyerstahl, P. 1994. Odor and structure. *Journal für Praktische Chemie/Chemiker-Zeitung*, 336:95–109.

Whitfield, F. 1998. Microbiology of food taints. *Int. Jour. Food Sci. Technol.*, 33:31–53.

Wichard, T., S.A. Poulet, C. Halsband-Lenk, A. Albaina, R. Harris, D. Liu, and G. Pohnert. 2005. Survey of the chemical defense potential of diatoms: Screening of fifty species for alpha, beta, gamma, delta -unsaturated aldehydes. *Jour. Chem. Ecol.*, 31:949–958.

Wium-Andersen, S., U. Anthoni, C. Christopherse, and G. Houen. 1982. Allelopathic effects on phytoplankton by substances isolated from aquatic macrophytes (Charales). *Oikos*, 39:187–190.

Wnorowski, A.U. 1992. Taste and odors in the aquatic environment: A review. *Water SA*, 18:203–214.

Wnorowski, A.U., and W.E. Scott. 1992. Incidence of off-flavors in South African surface waters. *Water Sci. Technol.*, 25:225–232.

Wu, J. 1988. Effect of environmental factors on geosmin production by *Fischerella muscicola*. *Water Sci. Technol.*, 20:143–148.

Wu, J.T., and F. Jüttner. 1988. Differential partitioning of geosmin and 2-methylisoborneol between cellular constituents in *Oscillatoria tenuis*. *Arch. Microbiol.*, 150:580–583.

Wu, J., P. Ma, and T. Chou. 1991. Variation of geosmin content in *Anabaena* cells and its relation to nitrogen utilization. *Arch. Microbiol.*, 157:66–69.

Yagi, M., S. Nakashima, and S. Muramoto. 1988. Biological degradation of musty odor compounds, 2-methylisoborneol and geosmin, in a bio-activated carbon filter. *Water Sci. Technol.*, 20:255–260.

Yano, H., M. Nakahara, and H. Ito. 1988. Water blooms of *Uroglena americana* and the identification of odorous compounds. *Water Sci. Technol.*, 20:75–80.

Young, W.F., H. Horth, R. Crane, T. Ogden, and M. Arnott. 1996. Taste and odor threshold concentrations of potential potable water contaminants. *Water Res.*, 30:331–340.

Zaitlin, B., and S.B. Watson. 2006. Actinomycetes in relation to taste and odor in drinking water: Myths, tenets and truths. *Water Res.*, 40:1741–1753.

Zimba, P.V., C.P. Dionigi, and D.F. Millie. 1999. Evaluating the relationship between photopigment synthesis and 2-methylisoborneol accumulation in cyanobacteria. *Jour. Phycol.*, 35:1422–1429.

Zaitlin, B., S.B.Watson, J. Ridal, T. Satchwill, and D. Parkinson. 2003. Actinomycetes in Lake Ontario: Habitats and geosmin and MIB production. *Jour. AWWA*, 95:113–118.

Zimmerman, W.J., C.M. Soliman, and B.H. Rosen. 1995. Growth and 2-methylisoborneol production by the cyanobacterium *Phormidium* LM689. *Water Sci. Technol.*, 31:181–186.

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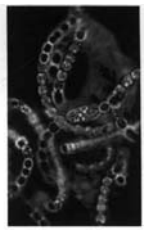

Chapter 16

Control of Cyanotoxins in Drinking Water Treatment

Gregory W. Harrington and Ryan Swank

Cyanobacteria, or blue-green algae, have long been known by water utility professionals for their adverse impacts on water quality. Problems include the reduction of water production due to filter clogging, the formation of taste and odor compounds, and the increase in naturally occurring organic matter and thus the formation of disinfection by-products. In addition, the formation of algal blooms may deem lakes or reservoirs unsuitable for swimming or boating and contribute to the depletion of dissolved oxygen, which may cause fish kills. Some species of cyanobacteria produce chemical toxins, and blooms of these cyanobacteria are commonly called "harmful algal blooms." The toxins are generally referred to as "cyanotoxins." Cases of livestock and wildlife poisoning due to harmful algal blooms have been reported since 1878 (Francis 1878). Adverse health effects are usually observed after ingestion of water near heavy algal blooms.

The purpose of this chapter is to focus on strategies used for control of cyanotoxins. The strategy to be used is dictated by a number of factors, including

- Which cyanotoxin needs to be removed,
- Whether the cyanotoxin is present within cyanobacteria cells or present outside of the cyanobacteria cells,
- The cyanotoxin concentration that is present in the source water,
- The target cyanotoxin concentration at the entry point to the distribution system,
- Technical capabilities of the control strategy.

As such, this chapter is organized into sections for each of the above items.

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PHYSICOCHEMICAL CHARACTERISTICS OF FRESHWATER CYANOTOXINS

Results from a USEPA meeting in May 2001 identified microcystin-LR, microcystin-YR, microcystin-LA, microcystin-RR, anatoxin-a, and cylindrospermopsin as the toxins of highest priority based on four criteria: health effects, occurrence in the United States, susceptibility to drinking water treatment, and toxin stability (USEPA 2001). The microcystins, anatoxin-a, and cylindrospermopsin have fundamentally different physicochemical attributes, and this has significant effects on the performance of cyanotoxin management strategies.

Figure 16-1 shows the general chemical structure of the microcystins, which have seven amino acids arranged in a cyclical structure and have molecular weights ranging from 900 to 1,200. The four microcystins mentioned in the previous paragraph have different amino acids inserted into the X and Z positions shown in Figure 16-1. The "Adda" group, common to all of the microcystins, is the part of the molecule that contributes most to the toxicity of the microcystins. Cylindrospermopsin has the molecular structure shown in Figure 16-2 and a molecular weight of 415. The uracil group of this molecule, the ring on the right end of the structure shown in Figure 16-2, is responsible for the toxicity of cylindrospermopsin. Anatoxin-a is a bicyclic secondary amine, as shown in Figure 16-3, and has a molecular weight of 165.

In general, molecules with higher molecular weight are more hydrophobic than molecules with lower molecular weight. The structures shown in Figures 16-1 through 16-3 are all relatively large, suggesting that these molecules are potentially susceptible to removal by technologies that target hydrophobic contaminants. However, all of the structures contain polar oxygen and nitrogen-containing moieties that also influence hydrophobicity. In general, the more of these polar groups in a molecule, the less hydrophobic the molecule is. Because each toxin has a unique molecular weight and a unique number of polar moieties, each toxin has its own degree of hydrophobicity. Therefore, each toxin will have a unique susceptibility to removal, and engineering design and treatment plant operation must consider which of the toxins are expected to be present in a given water supply.

In addition to removal technologies, the toxins are also susceptible to chemical transformation by oxidants like ozone and chlorine. These oxidants attack electron-rich moieties such as activated aromatics and carbon-carbon double bonds. Chlorine can also attack amines. Such moieties are present in all of the structures shown in Figures 16-1 through 16-3. For example, the uracil group of cylindrospermopsin contains one activated carbon-carbon double bond and two amine groups. The reactivity of these moieties is influenced by adjacent chemical structures and, therefore, each toxin will have a unique susceptibility to attack by chemical oxidants. Again, the predominant conclusion from this is that engineering design and treatment plant operation must consider which of the toxins are expected to be present in a given water supply.

INTRACELLULAR CYANOTOXINS VERSUS EXTRACELLULAR CYANOTOXINS

The previous section focused on the molecular structures of the cyanotoxins and their possible influence on control strategies. However, that discussion only applies to cyanotoxins that are dissolved in the bulk water (i.e., extracellular cyanotoxins). As described in Chapter 3, cyanobacteria produce cyanotoxins within the cell and the toxins are generally not released to the bulk water unless the cell wall is ruptured. Thus, removal of toxins contained within cyanobacterial cells (i.e., intracellular cyanotoxins)

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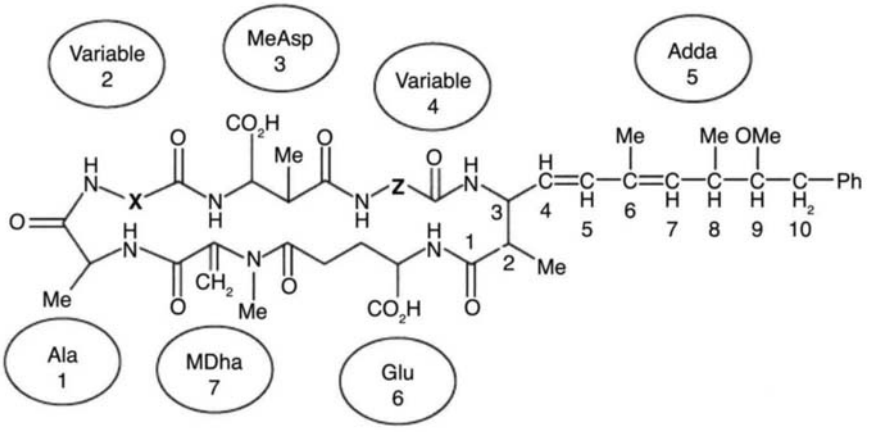

Figure 16-1 General chemical structure of the microcystins

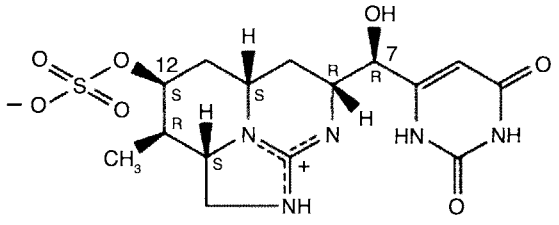

Figure 16-2 Chemical structure of cylindrospermopsin

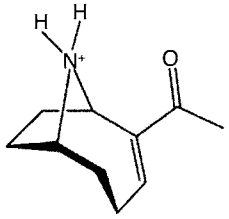

Figure 16-3 Chemical structure of anatoxin-a

will be dictated by the physicochemical properties of the cyanobacteria. Particle separation processes like conventional coagulation, flocculation, sedimentation, and filtration can be expected to remove intracellular cyanotoxins but not necessarily extracellular cyanotoxins. Oxidation processes may degrade extracellular cyanotoxins but could only degrade intracellular cyanotoxins if those toxins are released to the bulk water upstream of or within the oxidation process.

HEALTH-BASED GUIDELINES FOR CYANOTOXINS

Expectations for treatment process performance are dictated by both source water concentration and the desired concentration at the entry point to the distribution system. For example, if the source water cyanotoxin concentration is 10 μg/L and the target cyanotoxin concentration at the entry point is 1 μg/L, then the treatment process must

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achieve at least 90% removal of the cyanotoxin. This section summarizes the current state of drinking water guidelines and standards. These guidelines are used to illustrate target concentrations to be achieved at distribution system entry points. The following section describes the cyanotoxin concentrations commonly observed in drinking water sources.

There are no enforceable regulatory standards for the cyanotoxins in the United States, although the United States Environmental Protection Agency (USEPA) is considering required monitoring of the cyanotoxins as part of the Unregulated Contaminants Monitoring Rule. However, other nations and organizations have developed nonenforceable guideline values. For example, the World Health Organization (WHO) has adopted a provisional guideline value for microcystin-LR of 1.0 μg/L (WHO 2006). Australia has suggested maximum concentrations of 1.3 μg/L and 3 μg/L for microcystin-LR and anatoxin-a, respectively (NHMRC and ARMCANZ 2001). Humpage and Falconer (2003) have proposed a 1-μg/L guideline for cylindrospermopsin. Thus, it is reasonable for utilities to develop distribution system entry point goals that are on the order of 1 μg/L for either of the cyanotoxins.

In November 2006, the USEPA released draft toxicological characterizations of anatoxin-a, cylindrospermopsin, microcystin-LR, microcystin-RR, microcystin-YR, and microcystin-LA (USEPA 2006a, 2006b, 2006c). Surface water utilities are strongly encouraged to follow the final developments of these toxicological assessments.

CYANOTOXIN OCCURRENCE

A survey of water utilities in the United States and Canada confirmed the presence of microcystin-LR in 539 of 677 (80%) source water samples, with 4.3% of the samples having concentrations higher than the WHO guideline of 1 μg/L (Carmichael 2001). These results suggest that a small fraction of North American utilities need to remove microcystin-LR in order to achieve the WHO guideline. Of those samples exceeding the WHO guideline, concentrations ranged from 2 to 17 μg/L, suggesting that treatment processes need to achieve 50% to 95% removal of microcystin-LR.

A comprehensive survey of anatoxin-a and cylindrospermopsin occurrence in North American drinking water supplies has not been conducted. A survey of Dutch drinking water supplies revealed that anatoxin-a was detected at concentrations less than 1 μg/L in 33 out of 40 samples tested (Hoogenboezem et al. 2004). The maximum concentration detected was 12.1 μg/L. Burns (2004) reported that anatoxin-a was not detected in most samples collected during a survey of Florida waters, but one sample had an anatoxin-a concentration of 156 μg/L. Cylindrospermopsin was more commonly observed in these Florida waters, with concentrations typically in the range of 10 to 100 μg/L. The highest observed concentration was 202 μg/L. The author did not report whether the maximum concentrations were observed in water used to produce drinking water. Given these limited observations, cylindrospermopsin and anatoxin-a removals up to 99% might be needed in a drinking water treatment plant.

CYANOTOXIN CONTROL

Cyanotoxin control strategies may be grouped into several conceptual categories as follows:

- Source water control
  - Prevention of cyanobacterial blooms
  - Eradication of cyanobacterial blooms
  - Surface water avoidance or blending of groundwater with surface water

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  - Implementation of waste management strategies
- Water treatment between source and distribution system
  - Removal of intracellular toxins
  - Removal of extracellular toxins
  - Degradation of extracellular toxins

These strategies are described in the following sections.

Source Water Control

Before cyanotoxins ever reach the water treatment plant, measures can be taken to prevent their presence in the water source. These measures include preventing bloom formation, eradicating existing cyanobacterial blooms, and applying appropriate waste management techniques for the disposal of cyanotoxin-containing waste streams.

Bloom prevention. Prevention of cyanobacterial blooms would prevent human and animal exposure to cyanotoxins. Some methods to prevent cyanobacterial blooms include nutrient reduction, artificial mixing/aeration, pH control, fish population manipulation, and algaecide use prior to bloom formation. Nutrient reduction can be completed by diversion of nutrient-rich waters away from reservoirs or other surface water sources, minimizing the use of fertilizers containing phosphate, and increasing forestation and vegetation along surface waters (Fawell et al. 1993, Falconer 1993). If the source has already been heavily loaded with nutrients, such strategies may take extended time periods to achieve their goals.

Mixing by mechanical devices or aeration typically prevents phosphorus, iron, and manganese release from organisms in the surface waters and lowers the temperature in the euphotic zone (Fawell et al. 1993). However, when artificial mixing is used, reservoirs with extensive shallow areas may not have a sufficiently large mixing-depth/euphotic-depth ratio to inhibit growth (Steffensen et al. 1999). Phosphate can also be limited by aerating surface waters where stratification occurs, which takes phosphates from the sediments (Falconer 1993).

Manipulation of fish populations for cyanobacteria control is usually completed by introducing fish that eat phytoplankton or by reducing the number of fish that eat zooplankton. The latter can be accomplished by introducing fish that will eat the zooplankton-consuming fish. This manipulation will lower algae abundance in surface waters due to direct consumption of the algae by zooplankton or by fish that eat phytoplankton (Falconer 1993).

Bloom eradication. Algaecides like copper sulfate may be used to prevent the formation of cyanobacterial blooms or to eradicate cyanobacterial blooms after they have formed (Falconer 1993, Ownby and Odell 1988, Hawkins et al. 1985). If algaecides are used for eradication of an existing bloom, cell lysis will occur and may temporarily increase extracellular toxin levels. These extracellular toxins are more difficult to remove in treatment, and consideration should be given to allowing time for degradation of the released toxins in the source, which could take up to several weeks (Steffensen et al. 1999). Public health problems have occurred following the addition of copper sulfate to a source water. For example, a disease outbreak occurred at Palm Island, Australia, when copper sulfate was added to control an algal bloom containing *Cylindrospermopsis raciborskii*. The result was a release of cylindrospermopsin, which caused a number of human poisonings (Ownby and Odell 1988, Hawkins et al. 1985).

Microcystin degradation can also be completed by adding an aerobic degradation product such as barley straw to the surface water (Falconer 1993). This by-product has been found to prevent cell division in cyanobacteria and green algae. This is also

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speculated to reduce the growth rate of more complex plants, but this effect is speculated to be minimal. *Microcystis aeruginosa* CCAP 1450/6 was found to be inhibited up to 95% when greater than 2.7 g/m^3 of barley straw was added. More work is required to determine if barley straw addition has other effects in these surface waters (Fawell et al. 1993, Falconer 1993).

Surface water avoidance. Avoidance of surface waters that contain cyanobacterial blooms is a possible exposure prevention method. Water utilities may consider a permanent or temporary switch from surface water to groundwater if a groundwater supply is available. An example of temporary use might be use of groundwater during the time period during which cyanobacteria are actively growing. Caution should be used to ensure that the groundwater is not under the influence of a nearby cyanotoxin-containing surface water. Utilities may elect to derive all of their water from the groundwater supply or to blend the groundwater with treated surface water (Jurczak et al. 2005).

Waste management. Drinking water treatment plants have the potential to produce waste streams that contain significant quantities of cyanotoxins. For example, sludges from conventional surface water treatment may contain cyanobacterial cells that release toxins upon cell death (Drikas et al. 2001). Also, powdered activated carbon (PAC) used in such facilities may contain adsorbed cyanotoxins that are capable of desorption under certain conditions. These toxins can be contained if this waste is disposed of in landfills (Hitzfeld et al. 2000). Other options for sludge disposal are releasing sludges to the local wastewater treatment plant to be treated or by land-spreading the sludge on agricultural fields. The toxin may be released into the environment this way, but significantly high levels of cyanotoxins would have to be present in order for humans or animals to be exposed.

Water Treatment Methods

Water utilities have a number of options available for controlling cyanotoxins in drinking water treatment plants. However, as noted earlier, the performance of the treatment system is dictated by the properties of the toxin to be removed. For example, the conventional particle removal treatment train of coagulation, flocculation, sedimentation, and filtration can remove cyanobacteria cells and intracellular cyanotoxins, but this treatment train is not effective at removing dissolved, or extracellular, cyanotoxins. Adsorbents like PAC or granular activated carbon (GAC) can effectively remove dissolved cyanotoxins, but utilities need to consider the effects of competing adsorbates like natural organic matter (NOM) on process design.

This section begins with an overview of cyanotoxin removal in complete treatment process trains. This is followed by a review of some unit processes that are used within surface water treatment plants.

Complete process train. Karner et al. (2001) performed evaluations of four drinking water treatment plants located on Lake Winnebago in Wisconsin. In the first year of their study, samples were collected from the raw water and from the distribution system entry point. Three of the four treatment plants were examined in the second year of the study by collecting samples at various points within the treatment train. These samples were analyzed for total microcystin concentration, and no attempt was made to distinguish between intracellular microcystin and extracellular microcystin. Figure 16-4 shows a time-series of data from a conventional treatment plant during the summer of 1998, and Figure 16-5 shows data from several locations within the same treatment plant during the summer of 1999. Data in Figure 16-4 reveal the seasonal trend in microcystin concentration that was apparent at all four of the Lake Winnebago treatment facilities. At all four treatment plants, treatment was able to

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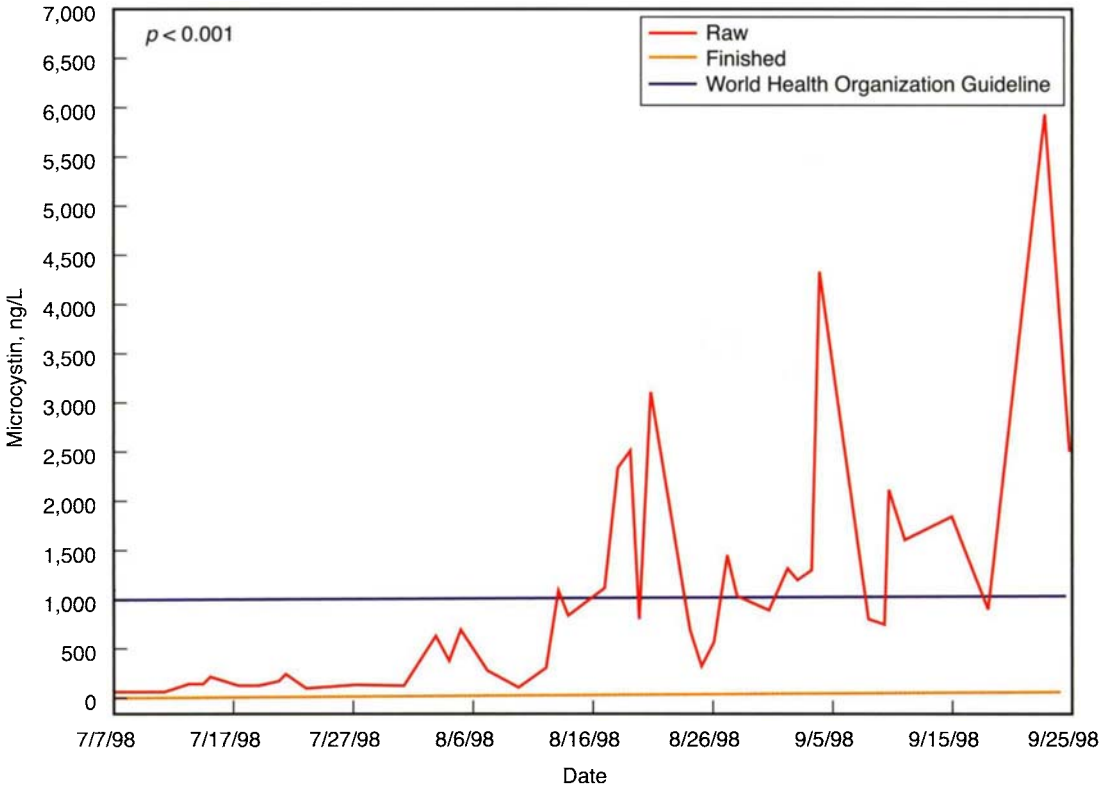

Figure 16-4 Microcystin concentrations for raw and finished waters at the Oshkosh, Wisc., water treatment plant during the summer of 1998

achieve the WHO guideline of 1 μg/L at the entry point to the distribution system even though this concentration was exceeded in the raw water source. Figure 16-5 was also illustrative of results obtained from the other two treatment plants. In all three cases, the most significant change in microcystin concentration occurred during the pretreatment stage. A complete characterization of chemical doses and residence times was provided by Karner et al. (2001) for each of the three treatment plants.

Subsequent studies in Australia, Poland, Germany, and Switzerland revealed similar results for microcystins, showing that both intracellular and extracellular microcystins were effectively removed by the process train (Hoeger et al. 2004, 2005; Jurczak et al. 2005). Jurczak et al. (2005) provided an extensive characterization of chemical doses and residence times within the two Polish water treatment plants. The reader is encouraged to obtain that paper as well as the paper by Karner et al. (2001) for assistance with operating conditions needed to achieve the observed removals.

Removal of cylindrospermopsin and anatoxin-a through various stages of operating drinking water treatment systems has not been reported in the peer-reviewed literature as of July 2007. However, Burns (2004) has reported cylindrospermopsin concentrations as high as 100 μg/L and anatoxin-a concentrations as high as 10 μg/L in treated drinking waters that were sampled in Florida. Removal of these cyanotoxins has been evaluated via some of the unit processes used in drinking water treatment. A review of these studies is provided in the following sections.

Coagulation/flocculation/clarification/filtration (conventional treatment). Several bench-scale studies have shown that conventional treatment with alum or

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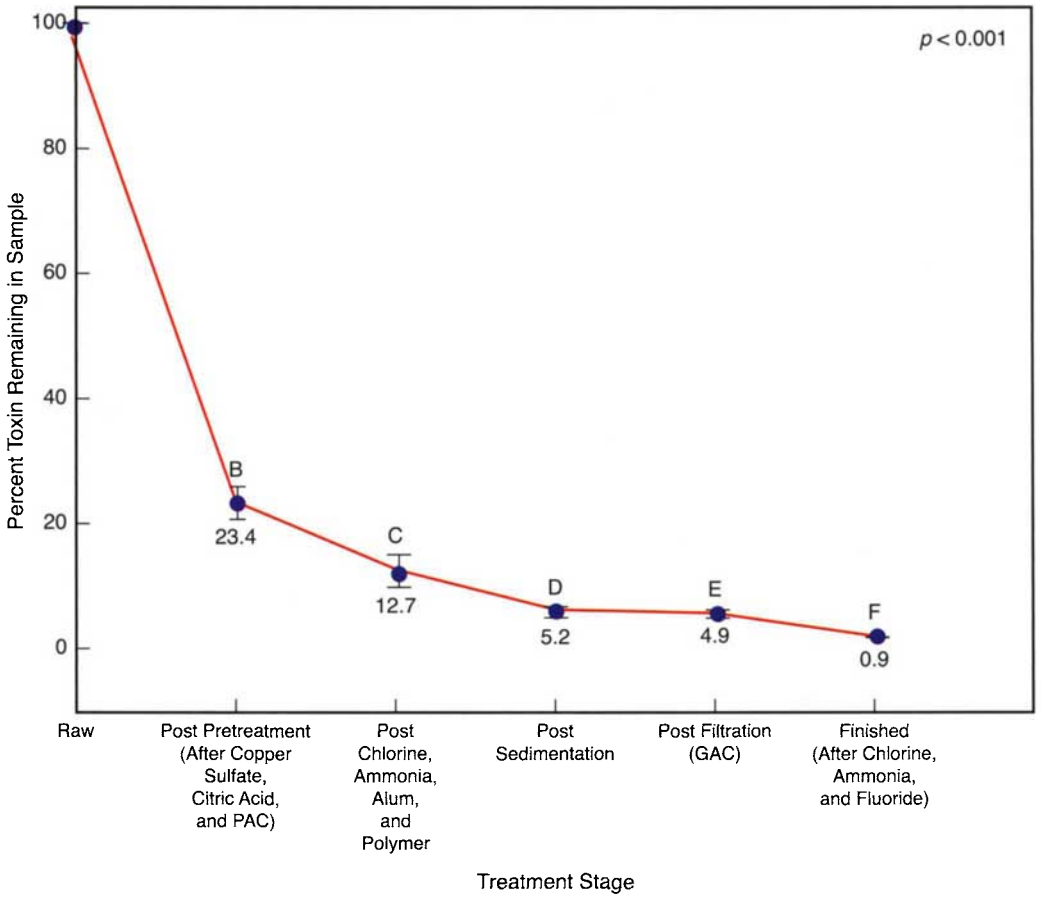

Figure 16-5 Average microcystin removals at different stages in the Oshkosh, Wisc., drinking water treatment plant during the summer of 1999. Error bars show standard deviations, and letters A through E show data points that are significantly different from each other at a 95% confidence level.

ferric chloride is ineffective at removing extracellular toxins produced by *M. aeruginosa* blooms (Wheeler et al. 1942, Hoffmann 1976). Himberg et al. (1989) observed similar results when testing the removal of uncharacterized extracellular hepatotoxic fractions of *M. wesenbergii*, *M. viridis*, and *Oscillatoria agardhii* using both alum and ferric chloride coagulation in combination with sand filtration and chlorination.

Keijola et al. (1988) studied the removal of extracellular anatoxin-a for process trains that included alum or ferric chloride coagulation, sand filtration, and chlorination. No removal was observed at an initial extracellular anatoxin-a concentration of 20 µg/L, but 14% removal and 49% removal were observed with alum and ferric chloride, respectively, at an initial extracellular anatoxin-a concentration of 200 µg/L.

However, Rositano and Nicholson (1994) and Hart et al. (1998) have reported up to 90% removal of intracellular microcystin using alum coagulation. Using coagulation with hydrated lime, sedimentation, and sand filtration, the Tomaszow-Lodz waterworks was able to reduce both intra- and extracellular microcystins to nondetectable levels on all but one day sampled (Jurczak et al. 2005). Studies have shown that the cyanobacteria cells can be removed in these processes without cell lysis and without release of toxins to the extracellular environment (Chow et al. 1998, 1999).

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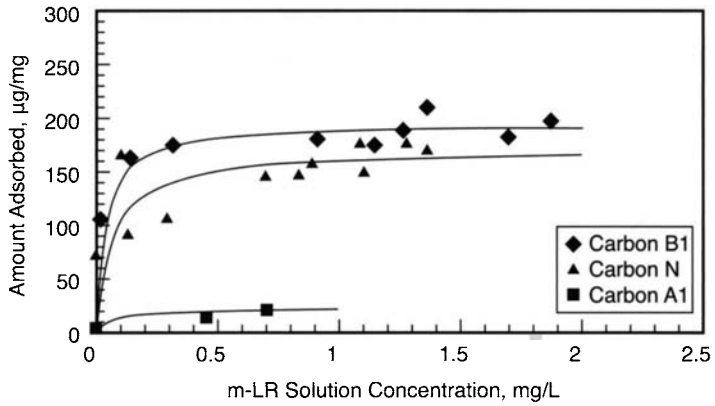

Figure 16-6 Adsorption of microcystin-LR onto activated carbon depends on the activated carbon used. In this example, two wood-based activated carbons achieved significantly greater removal of microcystin-LR than a coconut shell–based activated carbon. Removal of microcystin-LR was shown to depend on the volume of activated carbon pores that were larger than 0.7 nm in diameter. (Source: Pendleton et al. 2001.)

After a preoxidation step with ClO_2 (chlorine dioxide), addition of $FeSO_4$ (ferrous sulfate), $Al_2(SO_4)_3$ (alluminum sulfate), and PAC followed by sedimentation and dual-media filtration with sand and anthracite was generally able to remove remaining cell-bound and dissolved microcystins or reduce the total to less than 1.0 µg/L (Jurczak et al. 2005).

Biological treatment. Biologically active sand filters have been shown to reduce microcystin-LR concentrations to below the 1-µg/L WHO guideline according to both Bourne et al. (2006) and Ho et al. (2006a). This capability has been demonstrated at empty bed contact times common to both slow sand filtration and rapid sand filtration (Ho et al. 2006a). However, both studies were done under filtration rates common only to slow sand filtration, and it is unclear whether shear velocities under rapid sand filtration would have a significant impact on the bacterial community. Based on these two studies, it appears that an acclimation period of up to 6 days may be needed to achieve this level of performance. Bourne et al. (2006) showed that this acclimation period could be shortened by inoculation of known microcystin-LR degrading bacteria into the filter. To use this technology, a utility would need to operate their filters without a disinfectant residual present in the system.

Adsorption. *Powdered activated carbon.* PAC is typically added prior to or during coagulation in a drinking water treatment plant. With typical PAC doses ranging from 5 to 20 mg/L, PAC is commonly used in drinking water treatment to remove taste and odor causing compounds. Both extracellular microcystin-LR and extracellular anatoxin-a can be effectively removed by PAC addition, but results are highly dependent upon the type of PAC used (Hart et al. 1998, Pendleton et al. 2001). Figure 16-6 illustrates the adsorption capacity of three activated carbons for microcystin-LR. In this particular case, two activated carbons derived from wood have a stronger ability to remove extracellular microcystin-LR than an activated carbon derived from coconut shells. Because microcystin-LR has a relatively large molecular size, utilities should select activated carbons that have a relatively high percentage of pores with diameters larger than about 1 nm. Studies have also shown that the removal of microcystin-LR does not necessarily serve as an indicator for the removal of other microcystin species such as microcystin-LA (Newcombe et al. 2003). Finally, studies have also shown that competition by NOM also reduces adsorption capacity for cyanotoxins (Hart et al. 1998).

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Many studies have shown greater than 80% removal of microcystin-LR with varying initial microcystin-LR concentration, PAC dose, and type of PAC used (Keijola et al. 1988, Falconer et al. 1983, Donati et al. 1993, Lambert et al. 1994). For example, Donati et al. (1993) reported up to 85% removal of extracellular microcystin-LR at an initial concentration of 10 μg/L, using a 20-mg/L dose of wood-based PAC. PAC doses of 11 and 60 mg/L removed 90% (from 10 to 1 μg/L) and 98% (from 50 to 1 μg/L) of extracellular anatoxin-a, respectively (Bruchet et al. 1998).

Granular activated carbon. GAC is typically used in a fixed-bed column reactor, often as a medium in granular media filtration. Removal of extracellular cyanotoxins in GAC columns can occur by adsorption or by biodegradation due to the presence of microorganisms in the column. Intracellular cyanotoxins can theoretically be removed in these columns by filtration.

Carlile (1994) reported that extracellular microcystin-LR was effectively removed by GAC with a contact time of 15 minutes. Greater than 95% removal of extracellular microcystin-LR was possible at typical GAC column loading rates. However, competition with NOM reduces the capacity of adsorption and may lead to early breakthrough (Yoo et al. 1995).

It is likely that algal blooms will occur seasonally and, therefore, GAC beds may only be exposed to the algal toxins for a relatively short period of time. Hart et al. (1998) simulated the effect of GAC contact time on extracellular microcystin-LR removal when GAC was exposed to an extracellular microcystin-LR concentration of 10 μg/L for a period of 10 weeks. They found that longer contact times (>30 minutes) were required to minimize the peak concentration in the effluent and provide some level of biological treatment.

Algal toxins like extracellular microcystin-LR and extracellular anatoxin-a have been shown to be biodegradable and can be degraded in biologically active filters (Fawell et al. 1993). On the other hand, the formation of a biofilm may impair the ability of the GAC to adsorb extracellular toxins (Hitzfeld et al. 2000, Wang et al. 2007). When initial concentrations were below 0.15 μg/L, extracellular microcystin-LR was not removed by GAC in the presence of a biofilm or NOM (Lambert et al. 1996).

Hoffman (1976) found that GAC filtration could remove extracellular microcystin from water. As with PAC, GAC produced from wood was the most effective at cyanotoxin removal (Fawell et al. 1993, Mohamed et al. 1999). Fawell et al. (1993) also determined that column breakthrough was six times more rapid for a GAC produced from coconut than for GAC produced from either wood, peat, or coal (5,000 bed volumes versus 30,000).

Carlile (1994) also reported that extracellular anatoxin-a was effectively removed by GAC with a contact time of 15 minutes. Removal of extracellular anatoxin-a was better than removal of extracellular microcystin-LR. Greater than 95% removal of extracellular anatoxin-a was possible at typical GAC column loading rates. However, once again, competition with NOM reduces the capacity of adsorption and may lead to early breakthrough (Yoo et al. 1995). Falconer and Buckley (1989) found that breakthrough of acute toxicity (i.e., mouse bioassay via intraperitoneal injection) of concentrated extracellular anatoxin-a (0.1% by volume) occurred after 600 to 750 bed volumes of a pilot-scale sand filter that included 7 to 8 cm of GAC.

Membranes. Reverse osmosis (RO) excludes dissolved salts and organic components with a molecular weight of about 100 or higher by the use of a semipermeable membrane. Microcystin-LR and microcystin-RR have molecular weights near 1,000 and, as a result, have been retained in RO processes at above 95%. It should be noted that this process has a high contaminant retention level, which may cause problems with disposal of retained water that contains a high concentration of microcystin-LR (Lawton and Robertson 1999).

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Nanofiltration membranes are also capable of removing dissolved contaminants. Teixeira and Rosa (2006) concluded that 98% rejection of microcystin-LR and 95% rejection of anatoxin-a could be achieved when nanofiltration membranes were used. Rejection of microcystin-LR was attributed to size exclusion, while rejection of anatoxin-a was attributed to a combination of size exclusion and charge effects. Variation of feed water quality (total organic carbon and competing toxins), pH, and water recovery rate had no effect on cyanotoxin removal.

Studies with microfiltration and ultrafiltration membranes show that removal of intracellular toxins is feasible without significant cell lysis and its associated release of toxins to the extracellular environment (Chow et al. 1997, Gijsbertsen-Abrahamse et al. 2006). These membranes cannot achieve reliable rejection of extracellular cyanotoxins because the molecular weight cutoff of these membranes is larger than the molecular weights of the toxins.

Oxidation. Many studies have shown that oxidation can effectively degrade dissolved cyanotoxins. However, if applied to raw water containing toxic cyanobacteria cells, it is possible that the oxidant will be consumed in reactions with the cells. These reactions could cause cell lysis and toxin release. Therefore, an insufficient amount of oxidant may be available to react with the released toxins. For example, previous work has shown that the effectiveness of oxidation is reduced in the presence of live cyanobacterial cells (Rositano and Nicholson 1994). Therefore, it is best to use oxidation to remove dissolved cyanotoxins after the initial removal of algal cells by a set of processes like coagulation, flocculation, sedimentation, and filtration (Hart et al. 1998). The remainder of this section focuses on the individual oxidants used in drinking water treatment systems.

Free chlorine. Chlorine dissolves in water to form hypochlorous acid (HOCl) and hypochlorite. The concentration of hypochlorous acid increases with decreasing pH while the concentration of hypochlorite decreases with decreasing pH. At pH values less than 7.5, most of the chlorine dissolves to form hypochlorous acid. Hypochlorous acid has been established as a more effective disinfecting agent than hypochlorite, but this does not necessarily hold for all chemical oxidation reactions.

Oxidation of microcystin-LR by free chlorine is strongly dependent on pH, as illustrated in Figure 16-7. Clearly, the rate of microcystin-LR oxidation increases with decreasing pH, consistent with the rate of microorganism inactivation. At pH 6.0, the rate of microcystin-LR oxidation is 20 times faster than at pH 9.0.

Several investigators have established rate constants for a second-order reaction rate between free chlorine and microcystin-LR. Xagoraraki et al. (2006) determined that microcystin-LR reacts with hypochlorous acid with an average rate constant of 0.077 L/(mg · min). For hypochlorite, the average rate constant was observed to be 0.0036 L/(mg · min). On a molar basis, these rate constants are 91 $M^{-1}sec^{-1}$ and 4.3 $M^{-1}sec^{-1}$ for hypochlorous acid and hypochlorite, respectively. These compare to values of 120 $M^{-1}sec^{-1}$ and 6.8 $M^{-1}sec^{-1}$ reported by Rodríguez et al. (2007b).

In general, free chlorine concentrations are several orders of magnitude larger than cyanotoxin concentrations and, therefore, cyanotoxin oxidation occurs via a pseudo–first order rate law. This allows engineers to design and operate chlorination systems under the same CT concept that is used for microorganism inactivation. Xagoraraki et al. (2006) showed that a CT of 46 mg · min/L achieved an average of 95% microcystin-LR degradation in laboratory-grade water at pH 6. CT values of 220 mg · min/L and 650 mg · min/L achieved an average of 95% microcystin-LR degradation in laboratory-grade water at pH 7.5 and pH 9.0, respectively. These CT values were found to be conservative for three natural waters in Wisconsin, two natural waters in California, one natural water in Colorado, and one natural water in Massachusetts. To achieve 95%

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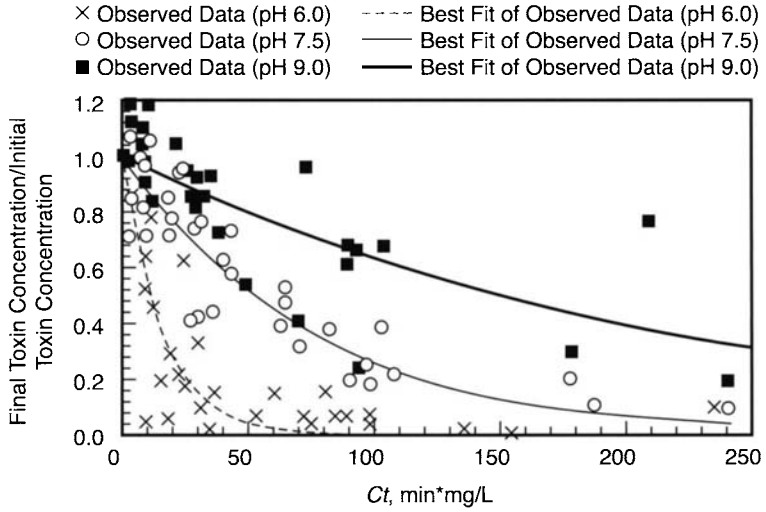

Figure 16-7 Influence of pH and CT value on inactivation of microcystin-LR by free chlorine (Reprinted from *Journal of Water Supply: Research and Technology–Aqua* 55(4):233–345, with permission from IWA Publishing)

microcystin-LR degradation with a 95% level of confidence, the CT values reported in this paragraph should be multiplied by a factor of approximately 9.

Studies with different microcystin species show that chlorine reactivity may or may not be species dependent (Acero et al. 2005, Ho et al. 2006b). Of the four species tested in the study by Ho and coworkers, microcystin-YR was the easiest to degrade, followed by microcystin-RR, microcystin-LR, and microcystin-LA. Rate constants for microcystin-RR were approximately twice as large as the rate constants for microcystin-LR and microcystin-LA. The study by Acero and coworkers concluded that there was little difference in chlorine reactivity among the different microcystin species. Ho and coworkers suggested that this observation may be linked to substances present in the water, such as NOM.

At pH 7, the rate of cylindrospermopsin degradation by free chlorine is approximately 10 times faster than the rate of microcystin-LR degradation (Rodríguez et al. 2007b). The cylindrospermopsin degradation rate decreases with increasing pH, likely due to the decreasing concentration of hypochlorous acid with increasing pH. At pH 10, the rates of microcystin-LR and cylindrospermopsin degradation are approximately the same. Interestingly, the rate of cylindrospermopsin degradation decreases with decreasing pH below pH 7.0. This occurs due to protonation of an amine group in the cylindrospermopsin molecule. Given these reported results, chlorination conditions that achieve 95% degradation of microcystin-LR should achieve more than 95% degradation of cylindrospermopsin.

To date, studies have shown that chlorine achieves poor degradation of anatoxin-a. Rositano and Nicholson (1994) reported using a chlorine dose of 15 mg/L for a 30-minute contact time and only removed 15% of extracellular anatoxin-a at an initial concentration of 20 μg/L in raw water and pH 7. Carlile (1994) and Hart et al. (1998) reported ineffective extracellular anatoxin-a removal by chlorination from pH 5 to 8. Similar results were reported by Rodríguez et al. (2007b).

Chlorination of intracellular cyanotoxins is generally discouraged because chlorination leads to cell lysis and release of cyanotoxins to the extracellular environment (Daly et al. 2007). Once cyanotoxins are released to the extracellular environment,

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degradation of the extracellular toxins by chlorine can occur. However, Daly and coworkers observed that the rate of toxin release was three times greater than the degradation of the released toxin. Thus removal of cells prior to chlorine addition is recommended.

Some have questioned whether toxic disinfection by-products are produced by reactions between chlorine and cyanotoxins. Tsuji et al. (1997) and Banker et al. (2001) have performed such studies but have needed to use molar cyanotoxin-to-chlorine ratios near 1:1 in order to detect and identify the by-products produced. In practice, these ratios are typically on the order of 1:1000 or smaller. In both studies, the by-products were found to be less toxic than the original cyanotoxins. Practitioners need to recognize that cyanotoxins are likely to be present at concentrations significantly smaller than NOM that produce trihalomethanes and haloacetic acids. Thus, utilities are likely to be more concerned with by-products of reactions between chlorine and NOM than they will be with reactions between chlorine and cyanotoxins. Utilities that increase CT values to enhance cyanotoxin removal need to be aware of the possible increase in trihalomethane and haloacetic acid formation.

Chloramines. Chloramination was found ineffective by Nicholson et al. (1994) with the addition of 30 mg/L monochloramine and a 5-day contact time in the degradation of microcystins. Both Hart et al. (1998) and Yoo et al. (1995) reported that chloramines were ineffective for the oxidation of extracellular anatoxin-a. Banker et al. (2001) also showed that cylindrospermopsin degradation was insignificant in the presence of chloramines.

Chlorine dioxide. Chlorine dioxide does not appear to be an effective oxidant to remove microcystin-LR, anatoxin-a, or cylindrospermopsin (Rodríguez et al. 2007b). A study by Hart et al. (1998) indicated that chlorine dioxide was ineffective at removing intracellular and extracellular microcystin-LR from a raw water source in the United Kingdom. When applied to treated water, a dose of 6 mg/L (the maximum dose allowed in the United Kingdom) or greater was required to remove at least 80% of extracellular microcystin-LR. A later study conducted by Kull et al. (2004) concluded that the overall rate constant for the reaction of microcystin-LR was less than that of hypochlorite, suggesting chlorine dioxide is not suitable for the degradation of microcystins. With increasing pH, the rate of chlorine dioxide reaction with microcystin-LR decreased.

Hydrogen peroxide. Hydrogen peroxide is an oxidizing agent that has had minimal use in drinking water treatment in the past. Lawton and Robertson (1999) showed that a 20-mg/L dose of hydrogen peroxide with a 60-minute contact time was only 17% effective in extracellular microcystin-LR degradation.

Advanced oxidation processes. Advanced oxidation processes are those that use hydroxyl radicals as oxidants. These radicals are generated by several methods including ozonation at high pH, ozonation combined with hydrogen peroxide, ultraviolet (UV) irradiation combined with hydrogen peroxide, and ferrous iron combined with hydrogen peroxide.

A study completed on a combined ozone/hydrogen peroxide treatment showed 100% removal of extracellular microcystin after 30 seconds (ozone dose, hydrogen peroxide dose, and the initial concentration of extracellular microcystin were not reported).

Research completed by Gajdek et al. (2001) determined that the combination of hydrogen peroxide and ferrous iron (the combination known as Fenton's reagent) resulted in nondetectable levels of extracellular microcystin-LR when added to a 300-μg/L microcystin-LR solution for 30 minutes.

Rodríguez et al. (2007b) reported half-lives of 5 minutes, 11 minutes, and 19 minutes for microcystin-LR, cylindrospermopsin, and anatoxin-a, respectively.

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Their summary suggests that ozone is more effective at cyanotoxin degradation than the advanced oxidation processes.

Ozone. The reaction of ozone with microcystin-LR occurs with a second-order rate constant of 4.1×10^5 $M^{-1}sec^{-1}$, roughly 1,000 times faster than HOCl (Rodríguez et al. 2007b). The reaction is independent of pH. The reaction of cylindrospermopsin with ozone is pH dependent. As the amine moieties in cylindrospermopsin become protonated at pH values less than 8.8, the reaction between ozone and cylindrospermopsin slows down. The reaction of ozone with the unprotonated form of cylindrospermopsin occurs with a rate constant of $\approx 2.5 \times 10^6$ $M^{-1}sec^{-1}$. For the protonated form of cylindrospermopsin, the reaction rate constant is five orders of magnitude slower at $\approx 4.0 \times 10^1$ $M^{-1}sec^{-1}$. Degradation kinetics of anatoxin-a by ozonation follow cylindrospermopsin behavior, with reaction rates slowing down as pH decreases below pH 9.4 and the amine group becomes more protonated. The second-order rate constants for reaction of ozone with the unprotonated anatoxin-a and the protonated anatoxin-a are $\approx 8.7 \times 10^5$ $M^{-1}sec^{-1}$ and 2.8×10^4 $M^{-1}sec^{-1}$, respectively.

CT values needed to achieve target levels of cyanotoxin degradation have not yet been reported. However, a number of studies provide some insights into the doses and reaction times needed. A study completed by Keijola et al. (1988) determined that addition of ozone at 1 mg/L had 100% degradation efficiency of extracellular microcystins at initial concentrations up to 60 μg/L. Rositano et al. (1998) showed that 99% degradation efficiency was achieved with an ozone concentration of 0.5 mg/L applied for 15 seconds. Hoeger et al. (2002) determined that degradation of a 10-μg/L microcystin-LR sample occurred within a 9-minute contact time with an ozone concentration of 0.5 mg/L. Rositano et al. (1998) reported 92% removal of anatoxin-a in 60 seconds with an initial anatoxin-a concentration of 20 μg/L and an ozone residual of 0.11 mg/L after this time. Anatoxin-a removal was slower than microcystin-LR removal using ozone but ozone was still effective.

Potassium permanganate. Rodríguez et al. (2007a) have summarized the interactions between permanganate and the cyanotoxins. The reaction rates are independent of pH for cylindrospermopsin and microcystin-LR. However, the reaction rate between permanganate and anatoxin-a decreases with decreasing pH between pH values of 8 and 10. At pH values outside this range, the rate of reaction between anatoxin-a and permanganate is independent of pH. Second-order rate constants are reported to be 3.6×10^2 $M^{-1}sec^{-1}$ for microcystin-LR, 4.3×10^4 $M^{-1}sec^{-1}$ for the unprotonated form of anatoxin-a, and 2.1×10^4 $M^{-1}sec^{-1}$ for the protonated form of anatoxin-a. The reaction rate between permanaganate and cylindrospermopsin is very slow, with a second-order rate constant $<1 \times 10^0$ $M^{-1}sec^{-1}$.

Although rate constants have not yet been used to develop CT values for cyanotoxin degradation by permanaganate, several studies have revealed some dose and contact time information. In a study with extracellular microcystin-LR, a dose of 1 mg/L of potassium permanganate removed 95% of microcystin-LR at an initial concentration of 200 μg/L in 30 minutes (Rositano and Nicholson 1994). Rodríguez et al. (2007a) concluded that dosages of 1.0 to 1.25 mg/L of potassium permanganate were sufficient to reduce microcystin concentrations to below the WHO guideline of 1.0 μg/L when used on a surface water that contained microcystin-LR concentrations of around 3.2 μg/L and a dissolved organic carbon concentration of 6.7 mg/L after 2 hours at pH 7.3.

Photocatalysis. Robertson et al. (1997) reported that a 200-μM solution of extracellular microcystin-LR was illuminated with a xenon UV lamp at 330 to 450 nm in the presence of a titanium dioxide (TiO_2) catalyst, resulting in a nondetectable level of microcystin-LR after a 40-minute contact time. Concentrations much lower than 200 μM resulted in nondetectable levels after a 2-minute contact time, which suggests

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that levels of microcystins in drinking water supplies would easily be degraded by this method.

Shephard et al. (2002) used a falling film semiconductor photocatalytic reactor to degrade extracellular microcystins. The reactor was operated by pumping the microcystin-containing solution as a falling film over a fiberglass sheet coated with TiO_2 as it was irradiated by a UV-C lamp. The water was recirculated within the reactor to continue microcystin degradation. This study determined that photocatalysis with the addition of a TiO_2 degrades microcystin-LR, -RR, and -YR.

Photocatalysis has also been studied for cylindrospermopsin degradation. Senogles et al. (2001) applied UV radiation at 262 nm with TiO_2 doses of 100 mg/L and 400 mg/L. The concentration of extracellular cylindrospermopsin ranged between 30 and 200 µg/L. The effectiveness of TiO_2 as a catalyst depended upon the type of TiO_2 product being used, the pH of the solution, and the temperature of the solution. Of the two different TiO_2 products used, the one with the larger crystal size was more effective. For both types of TiO_2, the most degradation of cylindrospermopsin occurred at pH 9. The effectiveness of one TiO_2 was not statistically affected when temperature was varied between 19° and 34°C. However, temperature did have a statistically significant effect on the performance of the other TiO_2 product, suggesting that product selection is important. Senogles et al. (2001) also determined the reproducibility of this experiment to have less than a 10% difference for 63% of the duplicates tested.

Oxidation summary. Oxidation may be used to degrade extracellular cyanotoxins in drinking water supplies. Ozone reacts more quickly with microcystin-LR, cylindrospermopsin, and anatoxin-a than the other oxidants, but this does not necessarily imply that ozone is the most cost-effective solution, and utilities are encouraged to evaluate this on a site-specific basis. Free chlorine is a reasonable option for oxidation of microcystin-LR and cylindrospermopsin, particularly in the pH 7 to 8 range. Meanwhile, potassium permanganate is a reasonable option for microcystin-LR and anatoxin-a. A significant number of surface water utilities use both potassium permanganate and free chlorine for taste and odor control as well as microbe inactivation. This combination of oxidants can be a cost-effective strategy for control of all three of the cyanotoxins considered in this chapter. As always, utilities are encouraged to perform site-specific evaluations to determine which strategy makes the most economic sense.

SUMMARY

As noted at the beginning of the chapter, numerous strategies are available for cyanotoxin control, and the most cost-effective strategy to use will be dictated by site-specific conditions. The cyanotoxins are not currently regulated by the USEPA and, as a result, American utilities may be reluctant to move forward with cyanotoxin control measures until the regulatory picture begins to clear. However, the forward-looking utility should consider studies that elucidate the current performance of their treatment plants, particularly during times of the year when cyanotoxin concentrations are at their peak in the water supply. The public has ready access to WHO guidelines via the Internet, and utilities should be prepared to communicate with the public on how their treatment plant performs relative to these guidelines. If treated water has cyanotoxin concentrations that exceed published guideline values, then that utility is encouraged to consider interim measures designed to achieve those guidelines. If the USEPA ultimately develops enforceable standards that differ from the current guidelines, the utility can implement a more permanent solution at that time.

Cyanotoxin control, whether on an interim or permanent basis, should consider source water prevention strategies as well as treatment strategies. Nutrient controls in

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the watershed are a good long-term solution, but utilities need to recognize that it may take years for such measures to reach their optimal effectiveness because of the nutrient levels already loaded into the water supply. Within the treatment plant, utilities are encouraged to provide removal of intracellular cyanotoxins upstream of processes designed to remove extracellular cyanotoxins. Intracellular cyanotoxin removal may be achieved by particle separation processes like conventional filtration and microfiltration membranes. Extracellular cyanotoxin removal may be achieved by activated carbon adsorption, oxidation, biodegradation, nanofiltration, or reverse osmosis. Utilities need to conduct site-specific evaluations to determine which cyanotoxin control strategy makes the most economic sense.

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REFERENCES

Acero, J.L., E. Rodriguez, and J. Meriluoto. 2005. Kinetic reactions between chlorine and the cyanobacterial toxins microcystins. *Water Res.*, 39(8):1628–1638.

Banker, R., S. Carmeli, M. Werman, B. Teltsch, R. Porat, and A. Sukenik. 2001. Uracil moiety is required for toxicity of the cyanobacterial hepatotoxin cylindrospermopsin. *Jour. Toxicol. Environ. Health, Part A*, 62(4):281–288.

Bourne, D.G., R.L. Blakely, P. Riddles, and G.J. Jones. 2006. Biodegradation of the cyanobacterial toxin microcystin-LR in natural water and biologically active slow sand filters. *Water Res.*, 40:1294–1302.

Bruchet, A., F. Bernazeau, I. Baudin, and P. Pierronne. 1998. Algal toxins in surface waters: Analysis and treatment. *Water Supply*, 16(1/2):611–623.

Burns, J. 2004. CyanoHABs—The Florida experience. In *Proceedings of the international symposium on cyanobacterial harmful algal blooms*. Washington, D.C.: USEPA.

Carlile, P.R. 1994. *Further studies to investigate of microcystin-LR and anatoxin-a removal from water*. Report FRO458. Marlow, U.K.: Foundation for Water Research.

Carmichael, W.W. 2001. *Assessment of blue-green algal toxins in raw and finished drinking water*. Denver, Colo.: Awwa Research Foundation and AWWA.

Chow, C.W.K., S. Panglisch, J. House, M. Drikas, M.D. Burch, and R. Gimbel. 1997. A study of membrane filtration for the removal of cyanobacterial cells. *Jour. Water Supply: Res. Technol. Aqua*, 46(6):324–334.

Chow, C.W.K., J. House, R.M.A. Velzeboer, M. Drikas, M.D. Burch, and D.A. Steffensen. 1998. The effect of ferric chloride flocculation on cyanobacterial cells. *Water Res.*, 32(3):808–814.

Chow, C.W.K., M. Drikas, J. House, M.D. Burch, and R.M.A. Velzeboer. 1999. The impact of conventional water treatment processes on cells of the cyanobacterium *Microcystis aeruginosa*. *Water Res.*, 33(15):3253–3262.

Daly, R.I., L. Ho, and J.D. Brookes. 2007. Effect of chlorination on *Microcystis aeruginosa* cell integrity and subsequent microcystin release and degradation. *Environ. Sci. Technol.*, 41(12):4447–4453.

Donati, C.D., M. Drikas, R. Hayes, and G. Newcombe. 1993. Adsorption of microcystin-LR by powdered activated carbon. *Water Jour. Austral. Water Wastewater Assoc.*, 20(3):25–28.

Drikas, M., C.W.K. Chow, J. House, and M.D. Burch. 2001. Using coagulation, flocculation and settling to remove toxic cyanobacteria. *Jour. AWWA*, 93(2):100–111.

Falconer, I.R. 1993. *Algal toxins in seafood and drinking water*. Cambridge, Mass.: Academic Press.

Falconer, I.R., and T.H. Buckley. 1989. Tumor promotion by *Microcystis* sp., a blue-green algae occurring in water supplies. *Med. Jour. Austral.*, 150(6):351.

Falconer, I.R., M.T.C. Runnegar, and V.L. Huynh. 1983. Effectiveness of activated carbon in the removal of algal toxin from potable water supplies: A pilot-plant investigation. In *Technical papers, tenth federal convention of the Australian Water*

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and Wastewater Association. Sydney, Australia. St. Leonards, Australia: Australian Water Association.

Fawell, J.K., J. Hart, J.A. James, and W. Parr. 1993. Blue-green algae and their toxins—Analysis, toxicity, treatment and environmental control. *Water Supply*, 11(3/4):109–121.

Francis, G. 1878. Poisonous Australian lake. *Nature*, 18(1):11–12.

Gajdek, P., Z. Lechowski, T. Bochnia, and F. Kepcynski. 2001. Decomposition of microcystin-LR by Fenton oxidation. *Toxicon*, 39(10):1575–1578.

Gijsbertsen-Abrahamse, A.J., W. Schmidt, I. Chorus, and S.G.J. Heijman. 2006. Removal of cyanotoxins by ultrafiltration and nanofiltration. *Jour. Membr. Sci.* 276(1-2):252–259.

Hart, J., J.K. Fawell, and B. Croll. 1998. The fate of both intra- and extracellular toxins during drinking water treatment. *Water Supply*, 16(1/2):611–623.

Hawkins, P.R., M.R.C. Runnegar, A.R.B. Jackson, and I.R. Falconer. 1985. Severe hepatotoxicity caused by the tropical cyanobacterium (blue-green alga) *Cylindrospermopsin raciborskii* (Woloszynska) Seenaya and Subba Raju isolated from a domestic water supply reservoir. *Appl. Environ. Microbiol.*, 50(5):1292–1295.

Himberg, K., A.M. Keijola, L. Hiisvirta, H. Pyysalo, and K. Sivonen. 1989. The effect of water treatment processes on the removal of hepatotoxins from *Microcystis* and *Oscillatoria* cyanobacteria: A laboratory study. *Water Res.*, 23(8):979–984.

Hitzfeld, B.C., S.J. Hoger, and D.R. Dietrich. 2000. Cyanobacterial toxins: Removal during drinking water treatment, and human risk assessment. *Environ. Health Perspect. Suppl. 1*, 108(S1):113–122.

Ho, L., T. Meyn, A. Keegan, D. Hoefel, J. Brookes, C.P. Saint, and G. Newcombe. 2006a. Bacterial degradation of microcystin toxins within a biologically active sand filter. *Water Res.*, 40:768–774.

Ho, L., G. Onstad, U. von Gunten, S. Rinck-Pfeiffer, K. Craig, and G. Newcombe. 2006b. Differences in the chlorine reactivity of four microcystin analogues. *Water Res.*, 40(6):1200–1209.

Hoeger, S.J., D.R. Dietrich, and B.C. Hitzfeld. 2002. Effect of ozonation on the removal of cyanobacterial toxins during drinking water treatment. *Environ. Health Perspect.*, 110(11):1127–1132.

Hoeger, S., G. Shaw, B. Hitzfeld, and D. Dietrich. 2004. Occurrence and elimination of cyanobacterial toxins in two Australian drinking water treatment plants. *Toxicon*, 43:639–649.

Hoeger, S.J., B.C. Hitzfeld, and D.R. Dietrich. 2005. Occurrence and elimination of cyanobacterial toxins in drinking water treatment plants. *Toxicol. Appl. Pharmacol.*, 203:231–242.

Hoffmann, J.R.H. 1976. Removal of microcystis toxins in water purification processes. *Water SA*, 2(2):58–60.

Hoogenboezem, W., K. Blaauber, and A.J. Wagenvoort. 2004. *The occurrence of toxic cyanobacteria in some Dutch surface waters used for the production of drinking water*. Amsterdam: Association of Rhine Waterworks.

Humpage, A.R., and I.R. Falconer. 2003. Oral toxicity of the cyanobacterial toxin Cylindrospermopsin in male Swiss albino mice: Determination of no observed adverse effect level for deriving a drinking water guideline value. *Environ. Toxicol.*, 18:94–103.

Jurczak, T., M. Tarczynska, K. Izydorczyk, J. Mankiewicz, M. Zalewski, and J. Meriluoto. 2005. Elimination of microcystins by water treatment processes—Examples from Sulejow Reservoir, Poland. *Water Res.*, 39:2394–2406.

Karner, D.A., J.H. Standridge, G.W. Harrington, and R.P. Barnum. 2001. Microcystin algal toxins in source and finished drinking water. *Jour. AWWA*, 93(8):72–81.

Keijola, A.M., K. Himberg, A.L. Esala, K. Sivonen, and L. Hiisvirta. 1988. Removal of cyanobacterial toxins in water treatment processes: Laboratory and pilot-scale experiments. *Toxicity Assess.*, 3:643–656.

Kull, T.P.J., P.H. Backlund, K.M. Karlsson, and J. Meriluoto. 2004. Oxidation of the cyanobacterial hepatotoxin microcystin-LR by chlorine dioxide: Reaction kinetics, characterization, and toxicity of reaction products. *Environ. Sci. Technol.*, 38(22):6025–6031.

Lambert, T.W., M.P. Boland, C.F.B. Holmes, and S.E. Hrudey. 1994. Quantitation of the microcystin hepatotoxins in water at environmentally relevant concentration with the protein phosphatase bioassay. *Environ. Sci. Technol.*, 28(4):753–755.

Lambert, T.W., C.F.B. Holmes, and S.E. Hrudey. 1996. Adsorption of microcystin-LR by activated carbon and removal in full scale water treatment. *Water Res.*, 30(6):1411–1422.

Lawton, L.A., and P.K.J. Robertson. 1999. Physico-chemical treatment methods for the removal of microcystins (cyanobacterial hepatotoxins) from potable waters. *Chem. Soc. Rev.*, 28(4):217–224.

Mohamed, Z.A., W.W. Carmichael, J. An, and H.M. El-Sharouny. 1999. Activated carbon removal efficiency of microcystins in an aqueous cell extract of *Microcystis aeruginosa* and *Oscillatoria tenuis* strains

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isolated from Egyptian freshwaters. *Environ. Toxicol.*, 14(1):197–201.

National Health and Medical Research Council, and Agricultural and Resource Management Council of Australia and New Zealand (NHMRC and ARMCANZ). 2001. *1996 Australian Drinking Water Guidelines—Update 2001.* Fact Sheet 17A. Canberra, Australia: NHMRC.

Newcombe, G., D. Cook, S. Brooke, L. Ho, and N. Slyman. 2003. Treatment options for microcystin toxins: Similarities and differences between variants. *Environ. Technol.*, 24(3):299.

Nicholson, B.C., J. Rositano, and M.D. Burch. 1994. Destruction of cyanobacterial peptide hepatotoxins by chlorine and chloramines. *Water Res.*, 28(6):1297–1303.

Ownby, C.L., and G.V. Odell, eds. 1988. *Natural toxins: Characterization, pharmacology, and therapeutics.* New York: Pergamon Press.

Pendleton, P., R. Schumann, and S.H. Wong. 2001. Microcystin-LR adsorption by activated carbon. *Jour. Colloid Interface Sci.*, 240:1–8.

Robertson, P.K.J., L.A. Lawton, B. Munch, and J. Rouzade. 1997. Destruction of cyanobacterial toxins by semiconductor photocatalysis. *Chem. Commun.*, 1997(4):393–394.

Rodríguez, E., M.E. Majado, J. Meriluoto, and J.L. Acero. 2007a. Oxidation of microcystins by permanganate: Reaction kinetics and implications for water treatment. *Water Res.*, 41:102–110.

Rodríguez, E., G.D. Onstad, T.P.J. Kull, J.S. Metcalf, J.L. Acero, and U. von Gunten. 2007b. Oxidative elimination of cyanotoxins: Comparison of ozone, chlorine, chlorine dioxide, and permanganate. *Water Res.*, 41:3381–3393.

Rositano, J., and B.C. Nicholson. 1994. *Water treatment techniques for the removal of cyanobacterial toxins from water.* Report 2/94. Salisbury, Australia: Australian Centre for Water Quality Research.

Rositano, J., B.C. Nicholson, and P. Pierrone. 1998. Destruction of cyanobacterial toxins by ozone. *Ozone Sci. Eng.*, 20(3):223–238.

Senogles, P.J., J.A. Scott, G. Shaw, and H. Stratton. 2001. Photocatalytic degradation of the cyanotoxin Cylindrospermopsin, using titanium dioxide and UV irradiation. *Water Res.*, 35(5):1245–1255.

Shephard, G., S. Stockenstrom, D. de Villiers, W. Engelbrecht, and G.F.S. Wessels. 2002. Degradation of microcystin toxins in a falling film photocatalytic reactor with immobilizing titanium dioxide catalyst. *Water Res.*, 36(1):140–146.

Steffensen, D., M. Burch, B. Nicholson, M. Drikas, and P. Baker. 1999. Management of toxic blue-green algae (cyanobacteria) in Australia. *Environ. Toxicol.*, 14(1):183–195.

Teixeira, M.R., and M.J. Rosa. 2006. Neurotoxic and hepatotoxic cyanotoxins removal by nanofiltration. *Water Res.*, 40:2837–2846.

Tsuji, K., T. Watanuki, F. Kondo, M.F. Watanabe, H. Nakazawa, M. Suzuki, H. Uchida, and K. Harada. 1997. Stability of microcystins from cyanobacteria—IV. Effect of Chlorination on decomposition. *Toxicon*, 35(7):1033–1041.

US Environmental Protection Agency (USEPA). 2001. *Creating a cyanotoxin target list for the Unregulated Contaminant Monitoring Rule.* Cincinnati, Ohio: USEPA Technical Service Center.

US Environmental Protection Agency (USEPA). 2006a. *Toxicological reviews of cyanobacterial toxins: Anatoxin-a.* Cincinnati, Ohio: USEPA.

US Environmental Protection Agency (USEPA). 2006b. *Toxicological reviews of cyanobacterial toxins: Cylindrospermopsin.* Cincinnati, Ohio: USEPA.

US Environmental Protection Agency (USEPA). 2006c. *Toxicological reviews of cyanobacterial toxins: Microcystins-LR, RR, YR, and LA.* Cincinnati, Ohio: USEPA.

Wang, H., L. Ho, D.M. Lewis, J.D. Brookes, and G. Newcombe. 2007. Discriminating and assessing adsorption and biodegradation removal mechanisms during granular activated carbon filtration of microcystin toxins. *Water Res.*, 41(18):4262–4270.

Wheeler, R.E., J.B. Lackey, and S. Schott. 1942. A contribution on the toxicity of algae. *Public Health Rep.*, 57(45):1695–1701.

World Health Organization (WHO). 2006. *Guidelines for drinking-water quality*, 1st addendum to 3rd edition. Geneva: WHO.

Xagoraraki, I., G.W. Harrington, K.K. Zulliger, B.A. Zeier, W. Krick, D. Karner, J. Standridge, and J. Westrick. 2006. Inactivation kinetics of the cyanobacterial toxin microcystin-LR by free chlorine. *Jour. Environ. Eng. ASCE*, 132(7):818–823.

Yoo, R.S., W.W. Carmichael, R.C. Hoehn, and S.E. Hrudey. 1995. *Cyanobacterial (blue-green algal) toxins: A resource guide.* Denver, Colo.: Awwa Research Foundation and AWWA.

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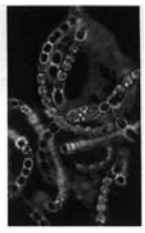

Chapter 17

Algae Removal Strategies

Kevin D. Linder and Kerry J. Meyer

Algae can cause several problems for water providers. The presence of algae and its effects can manifest as taste and odors (T&O) in the finished water, higher chemical costs, shorter filter run times, the presence of algal toxins, and an increase in disinfection by-products (DBPs). Algae pose treatment challenges such as increased dissolved organic carbon (DOC) that is difficult to coagulate and pH fluctuations that impact the effectiveness of water treatment chemicals. Algae are removed during clarification (e.g., sedimentation or flotation) and filtration during conventional treatment; its presence negatively impacts filter run times. Lower-concentration dissolved algal metabolites present a more complex water quality concern as they have the potential to pass through conventional treatment and can have health and aesthetic implications on the finished water quality. Over the past several years, there has also been an increased awareness of the adverse effects of algal toxins (such as microcystin). The World Health Organization (WHO) has developed a 1.0-µg/L guideline for finished water microcystin concentration. Historically, the perception was that algae only impacted the aesthetic qualities of finished drinking water. Now it is being recognized that source water algae control and a treatment plant's ability to remove algae from source water is an important step in delivering safe drinking water to the public. The focus of this chapter is the removal of algae through drinking water treatment. Source water algae control and the removal of algal metabolites are discussed elsewhere in this text.

Water treatment plants are given the challenge of removing algae from their source water while maintaining the particulate removal and inactivation requirements necessary to meet all the state and federal regulations. There are several approaches to this challenge of algae removal. Several factors need to be evaluated before determining the best approach for any given utility. These factors include raw water characteristics such as source type, source condition, climate, competing treatment priorities, water quality goals, and existing infrastructure. A complete characterization of the source water(s) must be completed before deciding on a treatment approach to address algae removal. The next step is to decide which available technology is the best fit for the application. The treatment goal should be the adoption of a treatment approach that provides the treatment plant operator with the tools to reliably meet all water quality goals day in and day out regardless of source water fluctuations, seasonal changes in algae populations and type, and even source changes.

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This chapter will separate algae removal strategies into groups, with the main treatment approaches being physical removal, chemical oxidation, and reservoir management, which often includes both physical treatment and oxidation. Advantages and disadvantages of each approach will be discussed as well, and water characteristics especially suited for a given approach will be highlighted. Regardless of the treatment process that is adopted, the most critical key to algae removal is to ensure that the treatment process selected is optimized during plant operation. One of the most important pieces to treatment plant optimization is the awareness (and sometimes anticipation of) changes in raw water quality and the ability to make treatment decisions to optimize plant performance in response. This understanding allows the operator to take a more proactive posture versus a reactive one. The more proactive the treatment process decisions are, the less chance there is for episodes of undesirable finished water quality (T&O events, etc.).

A treatability index will be discussed to aid utilities in providing the operators with real-time raw water quality data to make the best decision to minimize the impact of algae coming into the plant. The goal is to give the operator tools to succeed and make a commitment to treatment excellence.

TREATMENT PROCESSES

There are a variety of pathways for algae removal in water treatment. Reservoir management, pretreatment, physical removal through conventional treatment (or modifications thereof), oxidation, biological treatment, and adsorption techniques all have varying degrees of effectiveness on algae removal in water treatment.

Reservoir Management

Sometimes utilities find it most helpful to treat water at the source water reservoir before withdrawing it for drinking water treatment. Alternatives for source water management include chemical oxidation or the addition of algaecide such as copper sulfate (discussed in subsequent sections), aeration and/or mixing, use of alternative water intakes that retrieve water at the location of minimum algae concentration (usually the hypolimnetic zone), or pH adjustment (liming) in order to deter growth of certain pH-sensitive algae. These source water management strategies are often used in conjunction with the treatment plant alternatives discussed in this chapter. Often it takes a robust treatment strategy of multiple barriers in order to maintain finished water quality free of the negative aesthetic or water quality implications resulting from algal blooms.

Physical Pretreatment

Pretreatment processes can aid in the effectiveness of the treatment processes at the treatment plant. These pretreatment processes include roughing filters, microstrainers, and bank filtration. The processes are very effective at removing algal cells, high levels of turbidity, viruses, and protozoan cysts (Le Chevallier and Au 2004). These processes also effectively remove the algal cells without lysing the cells and releasing the toxins contained inside the cell walls.

Use of pretreatment steps targeted at algae removal may be potentially beneficial for drinking water plants for the following reasons:

- Reduced coagulant dose
- Longer unit filter runs (or reduced risk of membrane fouling)

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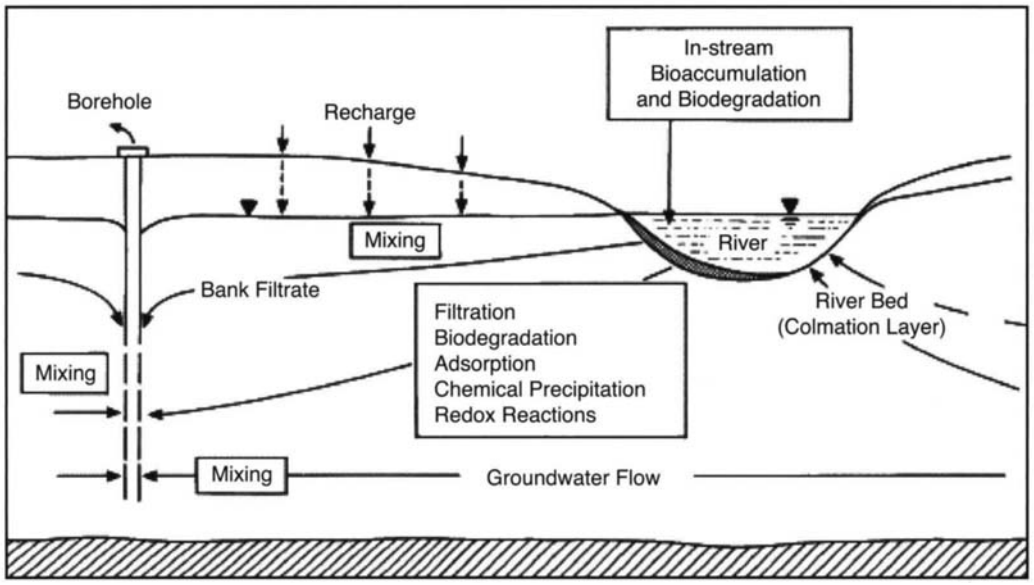

Figure 17-1 Riverbank filtration schematic (Source: Hiscock et al. 2002)

- Reduced risk of algae cells lysing and releasing algae toxins and T&O compounds
- Reduced disinfectant demand and/or DBP potential

River bank filtration. River bank filtration (RBF) has been around for a very long time and has demonstrated excellent performance in Europe for more than 100 years. From a cost-effectiveness standpoint, the amount of treatment realized by pulling the water through the bank is remarkable, not only for particulate removal but also for the reduction of natural organic matter (NOM), personal care products, endocrine disruptors, pharmaceuticals, and nitrate that are present in some source waters. It has been demonstrated that up to a 7-log removal of virus takes place in the first 30.48 m of travel distance. Given that the average size of algae is much greater than that of viruses, algae are likely easily removed by RBF. From a regulatory standpoint, specifically the Long Term 2 Enhanced Surface Water Treatment Rule (LT2), the source water characterization to determine bin size can be determined after the RBF. This is a huge benefit for utilities treating wastewater effluent–impacted source water. As summarized in Figure 17-1, in RBF there are several mechanisms at work including filtration or straining and biodegradation, as well as the adsorption or the accumulation of a dissolved substance on the surface of the material. There is also chemical precipitation as well as redox reactions taking place throughout the system. Algae, including cyanobacteria (blue-green algae), are effectively removed through this process on a daily basis through the filtration or straining portion of the system. This takes place in the first few feet of the bank filtration. RBF is an excellent first barrier in a multibarrier approach (e.g., RBF followed by conventional treatment, advanced oxidation process [AOP], biologically active filtration [BAF], and granular activated carbon [GAC]). This type of process selection emphasizes the importance of source water characterization. Next, a matrix of effective options for removal of the identified impurities must be developed. Finally, as with most approaches, it is helpful to perform bench-scale testing and/or piloting and use the data gathered to guide the process selection.

In a study of the Grand River in Ontario, Canada, algae and diatom removal ranged from 4.8 to 7.2 logs from the raw water to the collection well (Clancey and Stendahl

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Table 17-1 Removal rates of various algae

Organism	Type	Percentage Removal
Diatoms		
Cyclotella	Unicellular	10–70
Stephanodicus	Unicellular	10–60
Melosira	Filamentous	80–90
Synedra	Unicellular	40–90
Asterionella	Colonial	75–100
Fragilaria	Filamentous	85–100
Chlorophyceae		
Chlorella	Unicellular	10–50
Scenedesmus	Cenobia (4–8 cells)	15–60
Pediastrum	Cenobia (4–64 cells)	80–95
Blue-green algae		
Oscillatoria	Filamentous	40–50
Anabaena	Filamentous	50–70

1997). The data demonstrates the longer the flow path, the greater the removal. A study was performed on the Miami River in Cincinnati, Ohio, that estimated 1-log reduction for every 8.53 m of separation from the source (Gollnitz et al. 1997).

Roughing filter. A *roughing filter* is defined as a coarse media (typically rock or gravel) filter designed to remove larger diameter particles and improve downstream process performance. It can be applied before processes such as slow sand filtration, diatomaceous earth, or membrane filtration. Often the filter boxes are designed with separate areas containing media of decreasing particle size. Roughing filters can be successful at reducing raw water turbidity by 60% to 90%, depending on the influent water quality (quoted in LeChavallier and Au 2004). In a pilot study of a medium gravel (5.5-mm) horizontal roughing filter in Texas City, Texas, the filter removed 37% of the influent algal cells and 53% of the total chlorophyll. Filters ripened with algae actually removed more clay particles than the others (Collins et al. 1994). Additional water quality benefits such as increased removals of total organic carbon (TOC) and turbidity and unit filter run volumes were observed when alum was added upstream of the roughing filter (Ahsan et al. 1996).

Microstrainers. Microstrainers are made out of fabric meshes of stainless steel or polyester wires and can be used as a pretreatment step targeted at 15 to 45 μm (typically 30–35 μm). As a result of the mesh size, microstrainers are effective for removal of algal cells and large protozoans but not bacteria or viruses. Microstrainers can remove 40% to 70% of algae and 5% to 20% of turbidity (Mouchet and Bonnelye 1998). Table 17-1 summarizes the performance of microstrainers for the removal of various algae.

Physical Removal

Enhancements to the physical water treatment plant process are a common approach to algae removal optimization. This may include modifications to the conventional or direct filtration water treatment plant operation through the optimization of plant chemicals. It may also include upgrade of facilities to include processes like microsand enhanced flocculation, dissolved air flotation, or membranes.

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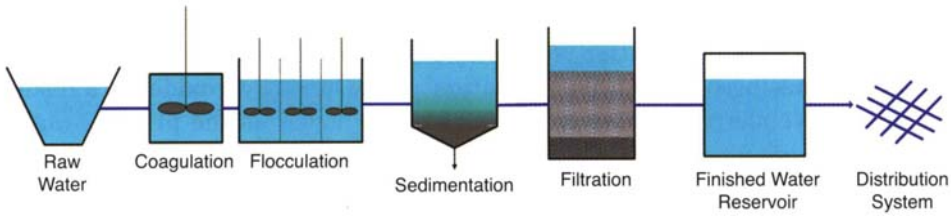

Figure 17-2 Conventional water treatment plant schematic (Courtesy of CH2M HILL)

Conventional Treatment

Modifications to conventional water treatment plant operation can have a significant impact on the success of algae removal through treatment. The following text summarizes how chemical selection, dosing, and overall approach can be optimized to target algae removal.

Overview. As shown in Figure 17-2, conventional treatment plants include the following steps in the treatment process: coagulation, flocculation, sedimentation, and filtration. The goal is to remove the algae cell intact without lysing the cell. This treatment train utilizes gravity to settle out floc particles in the sedimentation basin followed by the filtration of any unsettled particles in the filtration portion of the treatment process. The first defense in algae removal is coagulation. The coagulation process destabilizes the particle and provides the foundation for floc building and algae removal. The goal for the coagulation/flocculation process in a conventional treatment plant is to develop a floc that is heavy and will readily settle as the water slows down in the sedimentation basin. The question becomes how effective the different coagulants are at capturing the algae as part of the settleable floc.

One of the advantages of conventional treatment with respect to algae removal is that this treatment train will not lead to the release of intracellular toxins (Chow et al. 1998, 1999). However, the algae cells could release toxins as they decay within the sedimentation basin sludge if the sludge is not continuously removed (Chorus and Bartram 1999). The objective of the addition of chemicals within conventional treatment is to add enough positively charged aluminum or iron salts to neutralize the negatively charged colloids, including algae. At this neutralized state, floc particles will be able to increase in mass until they settle out of the water when the water velocity is reduced during sedimentation. Coagulant demand can be impacted by many things, such as the amount of dissolved NOM in the water (Edzwald and van Benschoten 1990, Edzwald 1993). Particles in the water can also be coated with adsorbed NOM (Tipping and Higgins 1982, Tiller and O'Melia 1993, O'Melia 1998, Buffle et al. 1998, Au et al. 1999). Therefore, enough coagulant must be added to neutralize the negative charges of both dissolved NOM and NOM that may be adsorbed on particle surfaces.

It is also important for operators to understand that "all algae are not charged equal"; that is to say that the charge density and zeta potential will vary from one type of algae to another. Even the charge on the same genus of algae can be different due to factors such as growth stage and pH (Ives 1956, 1959; Grunberg and Soeder 1967; Clasen et al. 2000). To the treatment process, this means that continual observation of the streaming current monitor and some type of floc particle measurement should be tracked and optimized. Studies have demonstrated that much more efficient algae removals can be realized at pH 6 vs. water of pH 7 or 7.5.

The effects were observed as part of a study that examined the effects of coagulation pH on the performance of a conventional treatment plant. At pH 6, the point of zero charge (PZC) was obtained at a ferric sulfate dose of 95 mg/L. The PZC was not

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achieved at the higher pH values. The scientists involved explained that the increase in pH impacts the solution chemistry Fe(III) by increasing the [OH^-] with each Fe(III) atom, thus increasing the precipitation rate of ferric hydroxide. As a result, the amount of positive charge per unit of added ferric is lower as the pH goes higher. The other explanation for the findings is that an increase in pH causes the surface charge of particles to become more negative which, in turn, requires more positively charged coagulant added to get to the PZC. There are also other things that the study indicated could impact the efficiency of the coagulation process, such as motility, presence of spines, or encapsulation in mucopolysaccharides (Briley and Knappe 2002).

One very accurate way of determining if PZC has been reached is by the use of the electrophoretic mobility data; most operators know this as zeta potential. It is the absolute value of the electric charge of particles within the water measured in millivolts. The PZC is theoretically achieved by the addition of positively charged coagulant to neutralize the negatively charged particles in the water and provide the zeta potential of zero. Another way to determine the PZC is using an instrument known as a streaming current detector (SCD). This instrument can either be used as a benchtop instrument or as an online analyzer. This instrument performs a titration on the water being sampled to determine electrical charge. The difference in the SCD is a value that is more qualitative due to the fact that the instrument can be "zeroed" by the operator when the plant is determined to be "optimized." At that point it is a relative value, subject to the next operator's definition of "optimized."

Algae size and shape can also impact how effectively algae are removed in the conventional treatment process. Small, spherically shaped algae are more easily removed than algae that have appendages or bristles that can reduce settling velocities (Bernhardt and Clasen 1991, 1994). In this case, enough coagulant must be added to fill in the gaps between appendages and bristles with floc. This emphasizes the importance of understanding the characterization of the targeted algae so the most effective algal removal approach can be implemented. Some algae can actually liberate themselves from floc, therefore requiring the addition of a preoxidant to arrest their mobility (Bernhardt and Clasen 1991, Petrusevski et al. 1993). Caution must be used so as not to overdose when using a preoxidation technique, as this method can cause the release of intracellular material or cell lysing if not done properly.

Coagulants. There are several coagulants used by water treatment plants in the United States. Aluminum sulfate (alum), ferric chloride, or ferric sulfate are some of the most common. The effectiveness of each differs depending upon several factors including algae type, water temperature, and pH. Many studies have been performed that clearly demonstrate the effectiveness of metal salts, organic polymers, and inorganic polymers for algae coagulation (e.g., Ives 1956, 1959; Tenney et al. 1969; McGarry 1970; Tilton et al. 1972; Konno and Sato 1986; Edzwald and Wingler 1990; Edzwald and Paralkar 1992; Bernhardt and Clasen 1991; 1994; Jiang et al. 1993; Briley and Knappe 1998; Knappe et al. 1998; Jun et al. 2001). The coagulation of algae occurs best when the surface charge, as discussed previously, gets to the neutral spot. This state is also referred to as the PZC. The floc size must also be sufficient to provide the density for it to settle. Which coagulant works best for any given conventional treatment process application is dependent on several factors. A natural coagulant derived from shrimp cells, chitosan, effectively removed greater than 90% of the algae in the water being tested (Chen et al. 1996).

Jar testing under several source water scenarios over the course of a year to capture seasonal variations in source water quality is a good way to determine which chemical regime works best. Traditionally, jar tests have been used for plant optimization using settled turbidity for the primary criteria. When focusing on algae removal,

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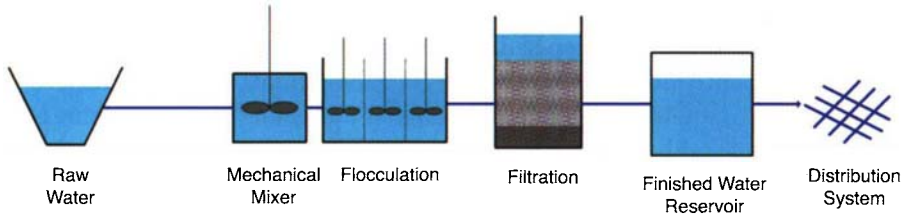

Figure 17-3 Direct filtration water treatment plant schematic (Courtesy of CH2M HILL)

it is helpful to use percent algae removal as the criteria. This will require some algae counting, but is well worth it to ensure optimized algae removal through the treatment process. This is important because turbidity measurements don't always correspond to algae counts.

Polymers. Polymers play an important role in the conventional treatment process by directly impacting flow settleability. Polymers have varying strengths of charge but potentially can reduce coagulant dose dramatically in achieving the PZC. Cationic, anionic, and nonionic polymers are available and work in different ways to improve floc settleability. Cationic polymers are used extensively as secondary coagulants and are used to neutralize particle charge in the rapid mix portion of the treatment train. They help to encapsulate algae in the floc. Varying degrees of charge are associated with different cationic polymers. Anionic polymers of high molecular weight can improve floc settleability and improve the amount of algae removed when used as a flocculant aid. The density of algae is close to the density of water. Floc particles containing algae have low densities. Consequently, to build floc of sufficient size to settle out may be in excess of 100 μm. To add strength to the larger floc, the addition of a high molecular floc can increase the "bridging effect" of the algae-laden floc to increase the settleability of the floc. Floc particles increase in size dramatically, resulting from the bridging mechanism by which high-molecular-weight anionic polymers work (Amirtharajah and O'Melia 1990). Bernhardt and Clasen (1991) demonstrated increased floc particle strength, which is especially helpful in removing nonspherical algae. Nonionic polymers have proven to be effective at algae removal by acting as a sticky agent for the floc particle building. Again, the object of floc building for conventional treatment is to develop a floc particle with enough mass to settle and the right chemical cocktail to encapsulate contaminants such as algae. Bench-scale testing should be used to determine the type of polymer to use as well as to determine the correct polymer dosing.

Direct Filtration

Modifications to direct filtration water treatment plant operation can have a significant impact on the success of algae removal through treatment. The following summarizes how the chemical selection, dosing, and overall approach unique to direct filtration can be optimized to target algae removal.

Overview. As shown in Figure 17-3, direct filtration is a treatment process very similar to conventional treatment except it lacks the sedimentation step in the process train. With the omission of the sedimentation step, the objective of the coagulation/flocculation process changes for operators. Instead of building a heavy, settleable floc, the direct filtration plant operator is building a very strong, filterable, pin floc. Despite the lack of sedimentation in the process, algae removals in direct filtration mode can be similar to those experienced in conventional treatment. The difference is that the algae removal takes place in the filtration portion of the process rather than in sedimentation.

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Deep bed filtration. One strategy that has proven effective for algae removal in a direct filtration plant is deep bed anthracite (~1.68 m of media) over a 25.4–30.48-cm sand layer. The anthracite media could also have slightly larger effective size to promote greater bed penetration. When optimized adequately, removals in excess of 95% are obtainable. Wemlinger Water Treatment Plant in Aurora, Colo., consistently experiences algae removals as high as 97%–99% utilizing dual-media filters configured as described above and operated in direct filtration mode.

Coagulants and polymers. As in conventional treatment, direct filtration plant operators add coagulants and polymers to achieve a PZC in the coagulation/flocculation step—the floc is just a much smaller size. As discussed in the conventional treatment section, many operators use zeta potential and or SCDs to aid in defining when the PZC is achieved. Zeta potential measures the electrical charge the water is carrying after chemical addition. Ideally, the target should be zero. However, running a water treatment plant is, many times, more of an art than science so what absolute charge is best for any given plant depends on what charge provides the highest level of algae removal. A streaming current monitor (SCM), or an SCD are online instruments that measure the same thing as the zeta potential, except the value is not absolute. The way these instruments work is a sample stream downstream from chemical addition is analyzed through the instrument continuously, and the charge is measured; however, with the SCM and SCD, the operator has the ability to "zero" the instrument when "plant optimization" has been achieved, then the plant, specifically coagulation, is maintained at this theoretical "zero." The problem with this relative determination of zero is that one operator's idea of zero may be different than that of the next operator. When optimizing for algae removal, it will be helpful to perform algae counts on the raw and finished water to find what zeta potential correlates to the highest percentage of algae removal. For the SCD and SCM, the algae counts of the raw and finished water allow the operator to "zero" his or her instrument when the highest percent algae removal is achieved through the process. Therefore, the same chemicals work for algae coagulation in direct filtration that work in conventional treatment: metal salts, organic polymers, and inorganic polymers. The difference is that in direct filtration, the chemical dose is an order of magnitude lower than in conventional treatment. For example, if the coagulant dose required to build settleable floc in conventional treatment is in the neighborhood of 30–40 mg/L alum, building a pin floc for the same water at a direct filtration plant would likely be as low as 3–4 mg/L. Common practice for direct filtration is to coagulate with a metal salt + cationic polymer, followed by the addition of a nonionic or anionic polymer as a filter aid to increase the "stickiness" of the floc as well as the strength. As always, the use of bench-scale testing will facilitate appropriate chemical selection for a given water treatment plant or source water. There are several disadvantages to direct filtration. The most obvious disadvantage is the lack of the sedimentation barrier. Direct filtration, with the shorter detention time, requires a higher disinfectant dose to make up for a small time (T) to obtain the required contact time (CT). The operator's reaction time to a change in raw water quality needs to be fast; complacency is magnified as quick reactions are a must with the shorter detention time.

Microsand Enhanced Flocculation

Microsand enhanced flocculation is a physical solid–liquid separation process; it is very similar to conventional treatment, and it incorporates rapid mix, coagulation, flocculation, sedimentation, and filtration, with the objective of using gravity and

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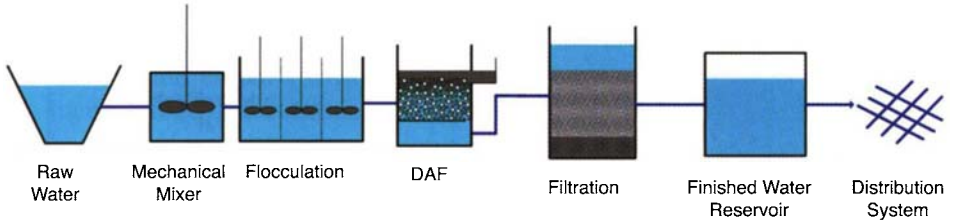

Figure 17-4 Dissolved air flotation water treatment plant schematic (Courtesy of CH2M HILL)

conventional water treatment plant chemicals to settle out raw water turbidity. The major difference is the use of microsand as a ballasting agent. The result is increased settling velocities and an increased overflow rate. The downside to this treatment process is an increase in operation and maintenance cost; power needs for this process are about two times as much as for conventional treatment. This technology is a good fit for utilities that are limited in space and existing infrastructure. Algae removal effectiveness is at least as good as conventional treatment. The Okeechobee Utility Authority Water Treatment Plant (Florida) utilizes the microsand ballasted flocculation process to treat a source high in TOC, cyanobacteria, and DBP precursors. Greater than 99% of the phytoplankton and 95% of the cyanobacteria were removed during clarification. Seventy-four percent of the total trihalomethane (TTHM) formation potential was reduced with 21% less aluminum sulfate in the coagulation process (Robinson and Fowler 2007). It is recommended that process performance be evaluated at pilot-scale before a final selection is made. There are several manufacturers of microsand enhanced flocculation processes that provide equipment and staff for piloting in order to determine if the process is appropriate and effective.

Dissolved Air Flotation

Dissolved air flotation (DAF) takes an alternative approach to algae (particulate) removal. Instead of creating settleable floc, the object of DAF is to attach the algae-laden floc to microbubbles introduced into the flocculation process as shown in Figure 17-4. The DAF coagulation process is consistent with the other solid–liquid separation techniques; coagulation focuses on neutralizing the surface charge of the particle. Like direct filtration, the difference between conventional floc and DAF floc is the floc diameter. DAF works best with pin floc as opposed to the much larger conventional floc size (hundreds of microns). Studies performed by Zabel (1985) found the removal of *Microcystis* was a log greater for DAF than for conventional treatment. Edzwald and Wingler (1990) demonstrated that DAF was also more effective at removing *Stephanodiscus* and *Aphanizomenon* than was conventional treatment. In Western Victoria, Australia, DAF is used by a treatment plant on Lake Wallace, a highly eutrophic lake with DOC levels typically in the 15–20-mg/L range and TOC in the 20–26 range. Levels of microcystin can be as high as 1 million cells/mL. This treatment plant uses a DAF process followed by ozone/biologically activated carbon (BAC). This treatment train removes 100% of the detectable T&Os.

A 1.8-log removal of the algae *Aphanizomenon* and *Microcystis* was achieved by pilot-scale DAF with similar results full scale (1.4–2.0-log removal) (Mouchet and Bonnelye 1998).

Given the many variables to consider when choosing an algae removal process, piloting and bench-scale testing become invaluable tools for technology selection.

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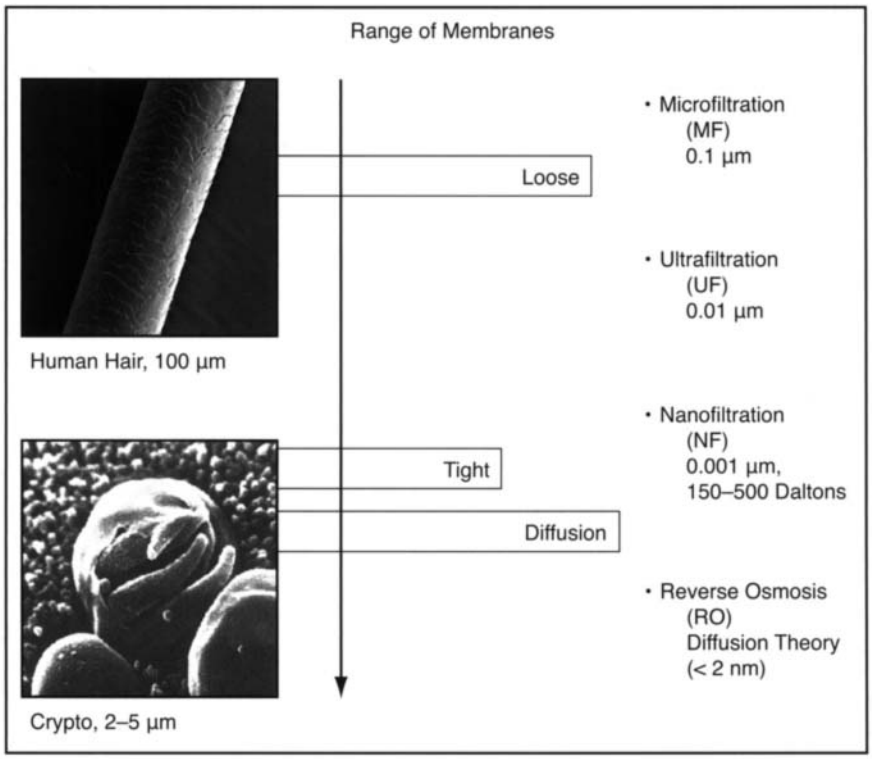

Figure 17-5 Membrane range and size exclusion (Courtesy of Kim Gill)

Membranes

Selection of membranes targeted for algae removal is a very straightforward process. If the membrane chosen has a smaller pore size than the minimum size of the targeted algae, the membrane will effectively remove the algae type. This is not usually a concern, since the minimum membrane size commonly used is 0.1 µm and the typical minimum algae size is 1.0 µm. There are some operations and maintenance costs involved, as well as several membrane configurations to choose from, before implementing this process. Including a pretreatment step in the design will often decrease the maintenance required on the membranes. The first step in planning a membrane system is to determine the membrane size required for both algae removal and removal of the other targeted contaminants. Next, the raw water characteristics will define the composition of the membrane. Raw water quality will also dictate if pretreatment, such as RBF, is required.

Membranes effectively remove algae if the correct pore size and membrane type are utilized. As shown in Figure 17-5, membrane size exclusion varies from 100 nm to less than 2 nm depending on the type of membrane selected. Individual membrane performance for algae removal will vary depending on the type of algae targeted. The membrane process can be enhanced with some type of pretreatment if desired. The disadvantage of membranes is that concentrate disposal options can be limited or expensive. Determining an appropriate brine disposal approach should be one of the first tasks undertaken when considering implementation.

Oxidation

Similar to enhancements to physical water treatment plant approaches, chemical oxidation is a common pathway explored for improved algae removal. Most experts agree that similar to physical treatment approaches, the goal of chemical oxidation

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is to oxidize the cell while avoiding lysing, if possible. Chemical oxidation can occur at the front end of the plant through the use of preoxidants for enhanced coagulation/flocculation or postfiltration during conventional primary disinfection. Due to their increased DBP formation potential, the performance of chlorine, ozone, and AOPs is based on the impact of oxidation for removal of algal metabolites during primary disinfection rather than preoxidation for improved coagulation/flocculation. For some utilities with low DOC water sources and good online instrumentation (in order to detect and avoid cell lysing), preoxidants like chlorine, ozone, and AOPs may have a positive impact on coagulation/flocculation of the algae without the negative water quality impacts (i.e., increased concentration of algae metabolites and DBPs) and should be explored on a case-by-case basis through bench- and/or pilot-scale testing. The impact of preoxidation with potassium permanganate and chlorine dioxide, however, is summarized below.

Potassium permanganate. Potassium permanganate ($KMnO_4$) is an oxidant that has been proven to have several excellent uses in water treatment and has demonstrated some degree of success at removing algae. The key to optimizing the use of $KMnO_4$ is providing adequate contact time. Whether the objective is to remove algae or to oxidize manganese, the key is to apply $KMnO_4$ as early as possible and maximize the time the chemical is in contact with the water. This may require installing a chemical feed system at the reservoir site. This can often be done at a pump station or valve house. Regardless of where the feed system is installed, the importance of providing adequate detention time cannot be overemphasized. Some $KMnO_4$ preoxidant applications have shown to be effective at removing particular types of algae. One study conducted by Chen and Yeh (2005) demonstrated that the addition of potassium permanganate increased the settling rate of the algae, and results were even more pronounced at low operating pH values (~4.0) and in the presence of Ca^{2+}. The invariance of zeta potential readings suggested that, for the water quality tested, doses of ≤3 mg/L did not have a significant impact on cell surface and likely did not lyse the algae cells. DOC concentrations did not vary with permanganate doses up to 3 mg/L, further validating the conjecture that the cells did not rupture. Therefore, with adequate contact time (1–5 hours), potassium permanganate can enhance algae removal through conventional treatment. An additional benefit of potassium permanganate over other preoxidants is that its addition may result in little or no increase in the formation of regulated DBPs, depending on the applied dose and the source water characteristics. Unfortunately, however, the addition of potassium permanganate has shown limited success with algae-associated 2-methylisoborneol (MIB) and geosmin, two of the most common algal metabolites.

Chlorine dioxide. Chlorine dioxide (ClO_2) is gaseous oxidant often applied as a primary disinfectant or as a preoxidant during conventional treatment. It is less effective at removing MIB and geosmin than some of the other oxidants. Lalezary et al. (1986) documented removals of 30% or less for geosmin and MIB with a ClO_2 dose of 2 mg/L. ClO_2 is effective at removing other odorous algae-related compounds such as 2,3,6-trichloroanisole (TSA), 2-isopropyl-3-methoxy pyrazine (IPMP), and 2-isobutyl-3-methoxy pyrazine (IBMP), with removals as high as 80%. The concern with the application of chlorine dioxide is the potential for the lysing of the cell and the release of the odorous intracellar material. This reinforces the importance of thorough bench-scale and pilot testing prior to full-scale implementation. The City of Loveland, Colo., found that increasing the preoxidant dose of chlorine dioxide upward of 0.8 mg/L did not decrease the T&O complaints they were addressing. After a bench-scale study, it was determined that *lowering* the preoxidant dose to 0.2 mg/L reduced the T&Os detected in the finished water. This dose of 0.2 mg/L was identified as the "threshold" chlorine dioxide dose that accomplished preoxidation just shy of the point of algae

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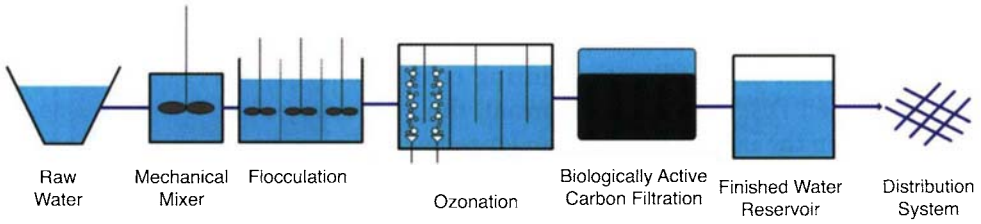

Figure 17-6 Ozone and biologically active carbon filtration plant schematic (Courtesy of CH2M HILL)

cell lysing. Chlorine dioxide does not appear to be effective at oxidizing cyanotoxins at doses used in typical water treatment processes (Yoo et al. 1995, Chorus and Bartram 1999, Hall et al. 2000) and therefore is not recommended for applications specifically targeted at oxidation of those compounds.

Chlorine. Chlorine (Cl_2) can only provide marginal effectiveness at oxidizing algal T&O compounds, such as MIB and geosmin, even with excessively high dosages and contact times (100 mg/L with a contact time of 100 hours) (Lalezary et al. 1986). However, chlorine has been successful at the bench level in removing cucumber odors associated with algae at a dose of 2 mg/L (Dietrich et al. 1995). Chlorine has also been proven effective at oxidizing some types of cyanotoxins. Chlorine was proven to be capable of oxidizing microcystin with a free chlorine residual of 0.50 mg/L for 30 minutes at a pH below 8 (Nicholson et al. 1994). There is concern that the by-products produced from the chlorination of cyanotoxins are toxic. Hitzfeld et al. (2000) reported that chlorination by-products of cyanotoxins can cause serious liver damage. The other by-product concern stems from the fact that the most efficient cyanotoxin removal occurs at low-pH and high-Cl_2 doses. This treatment scenario is likely to increase the formation of haloacetic acids.

Ozone. Numerous studies have shown that ozone (O_3) effectively removes MIB and geosmin. The key is the hydroxyl radical that is formed as O_3 decomposes. The effectiveness of O_3 for MIB and geosmin removal is dependent upon the ozone-to-TOC ratio; the higher the ratio, the better the removal. Lundgren et al. (1988) observed that high ozonation doses (7–20 mg/L) were accompanied by a substantial increase in off-flavor compounds identified as ozonation by-products. The other concern with feeding ozone is the potential to form bromate if the source water contains bromide. Bromate is an unwanted DBP that is a suspected carcinogen and is regulated at low-level concentrations (ng/L). Ozone is also very effective at removing some types of cyanotoxins. For example, ozone effectively removed microcystin (100%) at a dose of 0.50–1.1 mg/L (Chorus and Bartram 1999, Rositano et al. 2001). As shown in Figure 17-6, it is recommended that ozone be followed by biofiltration to increase the biostability of the water and decrease the likelihood of regrowth and increased chlorine demand in the distribution system. Ozone or the AOP followed by biofiltration typically lowers the DBP formation potential as well by removing organic precursors prior to disinfection.

In Western Victoria, Australia, there is a treatment plant on Lake Wallace that experiences challenging source water quality. Lake Wallace is a highly eutrophic lake with DOC levels typically about 20 mg/L and TOC in the 15–26 mg/L range. Levels of microcystin can be as high as 1 million cells/mL. This treatment plant utilizes DAF process followed by the ozone/BAC process (Hill and McMillan 1998). This treatment train has proven to effectively remove all detectable tastes and odors.

Advanced oxidation process. Like ozonation, the AOP utilizes the hydroxyl radical to oxidize algae. AOP combines ozonation with UV or hydrogen peroxide (H_2O_2), or by adding hydrogen peroxide and UV, as shown in Figure 17-7. All types of AOP are

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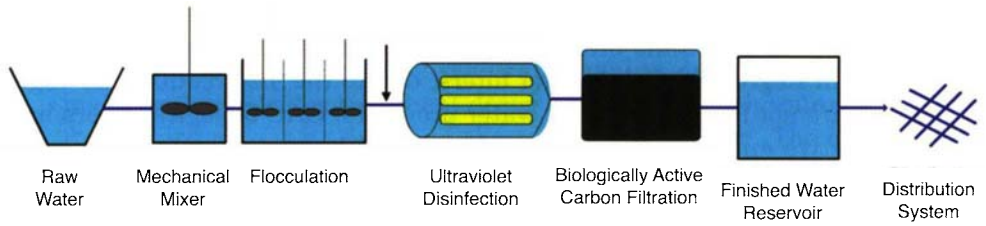

Figure 17-7 Ultraviolet AOP and biologically active carbon filtration plant schematic (Courtesy of CH2M HILL)

effective at removing most MIB and geosmin, with the combination of H_2O_2/UV being the least effective of the three. Geosmin appears to be oxidized more easily than MIB by AOPs (Glaze et al. 1990). H_2O_2/UV has been proven to effectively oxidize microcystin. As with ozone, it is recommended that AOP be followed by biofiltration to increase the biostability of the water and decrease the likelihood of regrowth and increased chlorine demand in the distribution system. Ozone or AOP followed by biofiltration typically lowers the DBP formation potential as well by removing organic precursors prior to disinfection.

An AOP treatment approach that utilizes ozone plus hydrogen peroxide is currently in place at the Okeechobee Utility Authority Water Treatment Plant. This plant uses the microsand-ballasted flocculation process to enhance the sedimentation process, which is followed later in the treatment train with a peroxone disinfection process. The peroxone disinfection process uses hydroxyl radicals produced during the spontaneous decomposition of ozone. The addition of hydrogen peroxide into the ozone contactor accelerates the decomposition of ozone, thus increasing the hydroxyl radical concentration and reaction rate. Peroxone has been proven effective at the reduction of cyanotoxins as well as T&Os (Robinson and Fowler 2007). One important consideration for implementing H_2O_2/UV AOP is the higher power use required to operate a UV system at the much higher dose for UV-AOP (compared to disinfection)—in the neighborhood of 750 mJ/cm^2.

Future approaches to oxidation. Chemical algae oxidation approaches on the horizon include combining techniques to take advantage of the strength of each method, resulting in a multibarrier approach to algae removal. There are already existing water treatment plants taking advantage of the synergistic effects of co-application of oxidants and combining treatment technologies to obtain the overall highest removal rates. One example of this multibarrier approach is practiced at the Okeechobee Utility Authority Water Treatment Plant and was discussed earlier in the chapter. This approach utilizes the strengths of both technologies to optimize algae removal.

Biological Treatment

Biological treatment is the engineered enhancement of microbiological populations native to the source water for the beneficial purpose of increased process performance. Many times biological treatment is implemented to decrease the biodegradable dissolved organic carbon (BDOC) in the water. In conventional treatment, implementing biological treatment can be as simple as moving the point of chlorination downstream of filtration. Due to the fact that MIB and geosmin are biodegradable along with many other T&O compounds, biological treatment is a viable option for these algae by-products and doesn't require the high operation and maintenance costs associated with ozonation or GAC. Biological filtration can be accomplished through many types of

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filter media; from slow sand filtration to deep-bed GAC filters that allow the media to go biological. Slow sand filtration is probably the most effective at removing MIB and geosmin. Regrettably, slow sand filters, due to the size required for high production rates, are not feasible for most water providers. GAC biological filters have proven to be effective and require a smaller footprint. The combination of ozonation and BAC filtration appears to be better at removing T&O compounds than GAC or ozone alone (Hrudey et al. 1995). It is recommended that biofiltration follow ozonation or AOPs to increase the biostability of the water and decrease the likelihood of regrowth and increased chlorine demand in the distribution system. Ozone or AOP followed by biofiltration typically lowers the DBP formation potential as well by removing organic precursors prior to disinfection.

Adsorption

In addition to physical removal, chemical oxidation, and biological treatment, the adsorption pathway is also commonly explored for improved algae removal. Adsorption can be evaluated through the use of PAC or GAC.

Powder activated carbon. PAC is a fine powdered carbon additive that is commonly used at the upstream end of a water treatment plant. It is added as a solid powder, often on an as-needed basis, in order to supplement ongoing treatment. One of the things PAC has proven to be moderately effective at is the adsorption of T&O compounds. PAC dosages of 12–40 mg/L have been proven to be effective at controlling MIB-related odors in natural waters. At doses greater than 5 mg/L, PAC is not a realistic option for direct filtration plants because the excess PAC would end up on the filters, resulting in dramatically reduced filter runs. Other organics in the water compete for adsorption sites, resulting in higher doses for water high in organics. Other factors impacting the effectiveness of PAC are contact time and source and composition of the PAC itself. Some PAC types are more effective than others at removing algae.

Granular activated carbon. GAC is used as a substitute for granular filter medium or as an additional process for the removal of organic compounds, including algal metabolites and T&O control. One of the limitations to GAC is that over time, the GAC becomes exhausted and requires replacement or regeneration, otherwise contaminant (i.e., taste and odor) breakthrough will occur. The fact that GAC requires periodic replacement or regeneration makes the operation and maintenance cost of GAC a serious consideration for water purveyors looking at this technology. Like PAC, GAC effectiveness is impacted by the amount of organics absorbed on the media and the composition or source of the media. Both PAC and GAC are oxidized when contact with an oxidant such as chlorine is experienced. Consequently, the feeding of $KMnO_4$ upstream of PAC is probably not a good treatment approach. GAC implementation requires a more engineered approach, as the empty-bed contact time (EBCT) is a critical parameter and needs to be sufficient for removing target algae. GAC is more quickly exhausted in highly organic water due to the competition for adsorption sites.

PLANT OPTIMIZATION

A critical component to plant optimization, regardless of treatment train type, is the optimization of the source water quality entering the treatment plant. As the water treatment industry becomes more technologically advanced and our understanding of the treatment process grows, those responsible for purifying the water need to ensure that the operators of the plant are given tools that provide them real-time raw water quality data and facilitate and streamline effective water treatment plant optimization.

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Treatability Index

Through the use of real-time raw water quality data, the operator is better able to make proactive water treatment decisions such as which source water to bring in and from which gate (e.g., depth) at the reservoir or stream. The question is, how does a utility build a tool to facilitate operator use of this real-time water quality data?

Utilities confront a common problem: their laboratories perform a multitude of analyses on their plant source waters, but the data generated rarely reach the operators until after the measured water parameters have changed. If an operator does see the data in time to make process changes, more than likely it is not in a format that can be related to the treatability of the water. Utilities face the challenge of getting laboratory data on source water quality to the operators in a timely, concise, and understandable format.

Presently, available technical descriptions of reservoir quality, such as the trophic state index (TSI), do not relate reservoir quality to treatability or do not address water treatment issues such as T&O. The TSI, developed by R.E. Carlson of the US Environmental Protection Agency, is a tool to indicate a lake's ability to support plant growth, as measured by phosphorus content, algal abundance, and depth of light penetration. The trick becomes understanding the data.

Aurora Water provides water for the City of Aurora, Colo. Water utility staff there investigated the issue of timely transfer of usable data from the laboratory to the operators. The goal was to improve and increase usage of valuable data generated in the laboratory to help optimize plant performance. Several options were explored, including giving operators copies of algal numeration and reservoir water quality data. That data provided limited insight because algae biomass is not reflected in an algae count, and if an operator isn't familiar with algae identification, the data could be confusing. Next, operations tried using chlorophyll *a*, a common measurement of green pigments found in plants, algae, and some bacteria, as an indicator of total algae biomass in the reservoir. Again, this indicated only a small part of the overall condition of the reservoir. Finding that none of the indices investigated served the purpose, operations and laboratory personnel collaborated to develop a tool that considered many reservoir parameters that affect treatment. The challenge became how to combine the effects of all these variables and present the information in a format that is comprehensive, easy to understand, timely, and concise.

The tool developed was named the treatability index (TI). Operations defined all the parameters that were critical to their treatment process. Next, they weighted the values according to relevance to treatment and combined them to produce two numbers reflecting treatability: one number addressed T&O, the other reflected filter clogging (FC) tendencies. Because algal mass and type affected treatment the most, the current TI uses parameters mostly related to algal mass and type. Figure 17-8 shows how these parameters were weighted by Aurora. In addition to chlorophyll *a*, operations personnel looked at historical T&O and FC problems with similar algae parameters, the effect of added mass of colonial algae, and the natural unit count of algae using a strip-counting technique on a Sedgewick–Rafter counting cell (a low-magnification, microscopic examination of organisms in water). Samples for algae enumeration are counted by counting at least three strips for each sample. Each strip is 50 mm long by .36 mm, which is the length of the Sedgewick–Rafter cell by the width of the Whipple grid. Also, they used a Secchi disk to determine water clarity and depth of visible light. A Secchi disk is a tool used to measure the clarity of water. It is basically a 22.86-cm circular disk divided into fourths. Two of the sections are colored white and two of the sections are colored black. The disk is lowered into the water to the point where it can no longer be observed. This distance is recorded as the Secchi depth.

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Parameters of Concern

Ranges and Weights

Filter Clogging Ratings		
Parameter	**Qualifier**	**Score**
Chlorophyll-*a*	0–2.9 ppm	0
	3.0–5.9 ppm	1
	6.0–8.9 ppm	2
	9.0 and above	3
Historical F.C. problems	No problems	0
with similar algae present	Moderate problems	1
	Many problems	2
Secchi depth	Greater than 10 ft	0
	Less than 10 ft	1
Multiple unit or very large	No	0
F.C. algae present	Yes	1
F.C. algal mass	Less than 500 per mL	0
	500–900 per mL	1
	1,000–2,999 per mL	2
	3,000 and above per mL	3
	Possible Total Rating of	10

Scoring

Figure 17-8 Example treatability index data sheet—filter clogging ratings (Courtesy of Aurora Water, with permission)

The quality control laboratory generates the treatability index data, scores them (as shown in Figure 17-9), and creates a report that includes other information related to each water source, such as a diagram depicting a withdrawal gate, a note on the depth of the photic zone, and concentrations of manganese and iron. Manganese and iron posed no treatment problems, so these parameters were not weighted or included in calculating the index. The TI also includes a small area for comments.

This index provides a quick overview of reservoir conditions with respect to the treatment process, using a comprehensive group of parameters to accurately reflect the source water. Operations staff uses this information to decide which source to use, whether to change withdrawal gates, or if it is necessary to prepare treatment for T&O. For example, if the TI shows source water "A" is experiencing a bloom of an algae type that has historically been a filter clogger or a source of taste and odors, the operator has a couple of options: If all other variables look good, the operator can switch withdrawal gates to a gate below the photic zone, or he or she can switch to a source that is not experiencing a bloom. Another example of how this tool can be used by the operator is for the operator to use the data in the TI to anticipate operational changes ahead of time to put him or her in a proactive rather than reactive mode.

If the operator sees on the treatability index a phosphorus spike in the supply reservoir, he or she can prepare the process for algae removal optimization, whatever that means to that plant. The real beauty of the treatability index is its flexibility. The parameters contained within the TI can be whatever water quality parameters the operators determine to be most critical. For example, a plant that has trouble with Mn can determine to have dissolved oxygen (DO) at the bottom of the reservoir put into the treatability index. The TI will quantify this parameter, and the operator can watch

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Taste and Odor Ratings		
Parameter	**Qualifier**	**Score**
Chlorophyll-*a*	0–2.9 ppm	0
	3.0–5.9 ppm	1
	6.0–8.9 ppm	2
	9.0 and above	3
Historical T&O problems	No problems	0
with similar algae present	Moderate problems	1
	Many problems	2
Secchi depth	Greater than 10 ft	0
	Less than 10 ft	1
Multiple unit or very large	No	0
T&O algae present?	Yes	1
T&O algal mass	Less than 500 per mL	0
	500–900 per mL	1
	1,000–2,999 per mL	2
	3,000 and above per mL	3
	Possible Total Rating of	10

Figure 17-9 Example treatability index data sheet—taste and odor ratings (Courtesy of Aurora Water, with permission)

the DO at the bottom of the reservoir and anticipate anoxia, which could mean $KMnO_4$ addition or a reservoir gate change to a higher gate within the water column that is not anoxic. Or, if a plant has trouble with a certain blue-green algae for taste and odor, as the operator sees these numbers getting to a historically troublesome level, he or she can increase the PAC dose to address it or move to a gate below the portion of the water column where these algae reside. This weighting can be changed or modified seasonally or as source water quality changes. For example, in spring when runoff depletes alkalinity and impacts the coagulation process, the operator could increase the weight of the alkalinity factor for 6–8 weeks or however long coagulation is impacted by alkalinity. However, in early fall, alkalinity is a nonfactor, and the operator may choose to remove alkalinity from the index altogether.

Aurora Water implemented the TI in the spring of 2000. The operations group immediately saw results from their reservoir quality guide that allowed operations staff to get a quick, quantitative, and understandable overview of the sources and their quality. The guide helps operators make informed decisions about raw water sources in a timely manner. Utilities can change and modify the weighting and parameters, as required. They can identify which parameters are most problematic and weigh them accordingly, as long as the parameters are consistent. For example, turbidity is relatively constant for Aurora Water; consequently, it is not included in the TI because turbidity is not a driving force on a daily basis in the selection of source water. If it were, turbidity could easily be included.

The treatability index is a tool to get data on source water quality to the operations staff in an understandable format that relates reservoir conditions to treatability. This tool is something that any utility can use to help optimize their treatment process and enhance communication between laboratory and operators (Benskin and Linder 2001).

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CONCLUSIONS

There are several approaches to the challenge of algal removal. These approaches include source water management, physical removal, adsorption, or chemical oxidation. The best approach for any given utility's algae removal needs should be selected based on several factors. These factors include raw water characteristics such as source type and source condition, as well as climate, competing priorities, water quality goals, and existing infrastructure. Next, bench-scale and pilot testing should be performed before full-scale implementation to determine the efficacy of the chosen process. Often it will depend on the frequency and the duration of the algal bloom with respect to what treatment technique is most practical. Currently, chemical oxidation and PAC are the most widely used treatment techniques, but some other treatment approaches, such as UV-AOP or ozone BAC, offer more proactive methods of algae metabolite control. Depending on the severity of the algae problem, the cost of these treatment alternatives may be offset by their consistent performance and multiple water quality benefits. Regardless of which treatment approach is chosen, operators must be provided with the tools and the culture to continuously improve and optimize the quality of water leaving the plant. There are tools, like the treatability index, available to improve communications between the lab and the operations staff that can be considered to assist with this.

REFERENCES

Ahsan, T., G.J. Alaerts, and J.P. Buiteman. 1996. Direct horizontal-flow roughing filtration. Part II: Performance and operational guideline. *Aqua*, 45(6):281–291.

Amirtharajah, A., and C.R. O'Melia. 1990. Coagulation processes: Destabilization, mixing, and flocculation. In *Water quality and treatment.* 4th ed. F.W. Pontius, ed. New York: McGraw-Hill.

Au, K.K., A.C. Pennison, S. Yang, and C.R. O'Melia. 1999. Natural organic matter at oxide/water interfaces: Complexation and conformation. *Geochim. Cosmoschim. Acta*, 63(19/20):2903–2917.

Benskin, P., and K. Linder. 2001. Treatability index provides operational guidelines. *Opflow*, 27(6):8–10.

Bernhardt, H., and J. Clasen. 1991. Flocculation of micro-organisms. *Aqua*, 40(2):76–87.

Bernhardt, H., and J. Clasen. 1994. Investigations into the flocculation mechanisms of small algal cells. *Aqua*, 43(5):222–232.

Briley, D.S., and D.R.U. Knappe. 1998. Optimization of coagulation conditions for the removal of algae. In *Proceedings of the AWWA annual conference.* Denver, Colo.: AWWA.

Briley, D., and D. Knappe. 2002. Optimizing ferric sulfate coagulation of algae with streaming current measurements. *Jour. AWWA*, 94(2):80–90.

Buffle, J., K.J. Wilkinson, S. Stoll, M. Filella, and J. Zhang. 1998. A generalized description of aquatic colloidal interactions: The three-colloidal component approach. *Environ. Sci. Technol.*, 32(19):2887–2899.

Chen, J., and H. Yeh. 2005. The mechanisms of potassium permanganate on algae removal. *Water Res.*, 39(18):4420–4428.

Chen, Y.M., J.C. Liu, and Y.H. Ju. 1996. Evaluation of chitosan for the removal of algae. In *Proceedings of the 4th international conference: The role of particle characteristics in the separation processes.* Jerusalem: Hebrew University of Jerusalem.

Chorus, I., and J. Bartram. 1999. *Toxic cyanobacteria in water.* London: E&FN Spon.

Chow, C.W.K., J. House, R.M.A. Velzeboer, M. Drikas, M.D. Burch, and D.A. Steffensen. 1998. The effect of ferric chloride flocculation on cyanobacterial cells. *Water Res.*, 32(3):808–814.

Chow, C.W.K., M. Drikas, J. House, M.D. Burch, and R.M.A. Velzeboer. 1999. The impact of conventional water treatment processes on cells of the cyanobacterium *Microsystis aeruginosa. Water Res.*, 33(15):3253–3262.

Clancy, J., and D. Stendahl. 1997. Groundwater or surface water—Microscopic evaluation of an Ontario River well system. In *Proceedings of the AWWA water quality technology conference.* Denver, Colo.: AWWA.

Clasen, J., U. Mischke, M. Drikas, and C. Chow. 2000. An improved method for detecting

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electrophoresis mobility of algae during the destabilization process of flocculation: Flocculant demand of different species and the impact of DOC. *Aqua*, 49(2):89–101.

Collins, M.R, C.M. Westersund, J.O. Cole, and J.V. Roccaro. 1994. *Evaluation of roughing filtration design variables*. Denver, Colo.: AWWA.

Dietrich, A.M., R.C. Hoehn, L.C. Dufresne, L.W. Buffin, D.M.C. Rashash, and B.C. Parker. 1995. Oxidation of odorous and nonodorous algal metabolites by permanganate, chlorine, and chlorine dioxide. *Water Sci. Technol.*, 31(11):223–228.

Edzwald, J.K. 1993. Coagulation in drinking water treatment: Particles, organics, and coagulants. *Water Sci. Technol.*, 27(11):21–35.

Edzwald, J.K., and A. Paralkar. 1992. Algae, coagulation, and ozonation. In *Chemical water and wastewater treatment II.* R. Klute and H. Hahn, eds. New York: Springer-Verlag.

Edzwald, J.K., and J.E. van Benschoten. 1990. Aluminum coagulation of natural organic matter. In *Chemical water and wastewater treatment.* H.H. Hahn and R. Klute, eds. Berlin: Springer-Verlag.

Edzwald, J.K., and B.J. Wingler. 1990. Chemical and physical aspects of dissolved-air flotation for the removal of algae. *Aqua*, 39:24–35.

Glaze, W.H., R. Schep, W. Chauncey, E.C. Ruth, J.J. Zarnoch, E.M. Aieta, C.H. Tate, and M.J. McGuire. 1990. Evaluating oxidants for the removal of model taste and odor compounds from a municipal water supply. *Jour. AWWA*, 82(5):79–84.

Gollnitz, W.D., F. Cossins, and J. DeMarco. 1997. Impact of induced infiltration on microbial transport in an alluvial aquifer. In *Proceedings of the AWWA water quality technology conference.* Denver, Colo.: AWWA.

Grunberg, H., and C.J. Soeder. 1967. Zetapotential zweier synchron kultivierter Chlorella-Stamme. *Arch. für Hydrobiol. Suppl.*, 33:331–362.

Hall, T., J. Hart, B. Croll, and R. Gregory. 2000. Laboratory-scale investigations of algal toxin removal by water treatment. *Water Environ. Jour.*, 14(2):143–149.

Hill, W., and R. McMillan. 1998. Design and operation of an ozone/BAC water treatment plant at Edenhope. In *Proceedings of the 61st annual water industry engineers and operators' conference.* Shepparton, Australia: Water Industry Operators Association Australia.

Hiscock, K.M., and T. Grischek. 2002. Attenuation of groundwater pollution by bank filtration. *Jour. Hydrol.*, 266(3-4):139–144.

Hitzfeld, B.C., S.J. Hoger, and D.R. Dietrich. 2000. Cyanobacterial toxins: Removal during drinking water treatment and human risk assessment. *Environ. Health Perspect.* (Suppl. 1) 108:113–122.

Hrudey, S.E., P.M. Huck, M.J. Mitton, and S.L. Kenefick. 1995. Evaluation of odor removal by pilot-scale biological treatment process trains during spring runoff in an ice-covered review. *Water Sci. Technol.*, 31(11):195–201.

Ives, K.J. 1956. Electrokinetic phenomena of planktonic algae. *Proc. Soc. Water Treat. Exam.*, 5:41–53.

Ives, K.J. 1959. The significance of surface charge on algae in water purification. *Jour. Biochem. Microbiol. Technol. Eng.*, 1(1):37–47.

Jiang, J., N.J. Graham, and C. Harward. 1993. Comparison of polyferric sulphate with other coagulants for the removal of algae and algae-derived organic matter. *Water Sci. Technol.*, 27(11):221–230.

Jun, H.B., Y.J. Lee, B.D. Lee, and D.R.U. Knappe. 2001. Effectiveness of coagulants and coagulant aids for the removal of filter-clogging *Synedra*. *Aqua*, 50:135–148.

Knappe, D.R.U., D.S. Briley, and N. Rastogi. 1998. Strategies for algae removal in conventional treatment. In *Proceedings of the AWWA annual conference.* Denver, Colo.: AWWA.

Konno, H., and A. Sato. 1986. Study for sand filter clogging by diatoms. *Water Supply*, 4:339–346.

Lalezary, S., M. Pirbazari, and M.J. McGuire. 1986. Oxidation of five earthy-musty taste and odor compounds. *Jour. AWWA*, 78(3):62–69.

LeChevallier, M., and K.K. Au. 2004. *Water treatment and pathogen control.* London: IWA Publishing.

Lundgren, B.V., A. Grimvalkl, and R. Savanhed. 1988. Formation and removal of off-flavor compounds during ozonation and filtration through biologically active sand filters. *Water Sci. Technol.*, 20(8/9):245–253.

McGarry, M.G. 1970. Algal flocculation with aluminum sulfate and polyelectrolytes. *Jour. WPCF*, 42(5):R191–R201.

Mouchet, P., and V. Bonnelye. 1998. Solving algae problems: French expertise and world-wide applications. *Aqua*, 47:125–141.

Nicholson, B.C., J. Rositano, and M.D. Burch. 1994. Destruction of cyanobacterial peptide

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hepatatoxins by chlorine and chloramines. *Water Res.*, 28(6):1297–1303.

O'Melia, C.R. 1998. Coagulation and sedimentation in lakes, reservoirs and water treatment plants. *Water Sci. Technol.*, 37(2):129–135.

Petrusevski, B., A. Vlaski, A.N. van Breeman, and G.J. Alaerts. 1993. Influence of algal species and cultivation conditions on algal removal in direct filtration. *Water Sci. Technol.*, 27(11):211–220.

Robinson, C., and S. Fowler. 2007. Fresh ideas for fresh water: Using innovative technology to obtain high-quality drinking water from Lake Okeechobee. *Fla. Water Res. Jour.*, 3:22–26.

Rositano, J., G. Newcombe, B. Nicholson, and P. Sztajnbok. 2001. Ozonation of NOM and algal toxins in four treated waters. *Water Res.*, 35(1):23–32.

Tenney, M.W., W.F. Echelberger Jr., R.G. Schuessler, and J.L. Pavoni. 1969. Algal flocculation with synthetic organic polymers. *Appl. Microbiol.*, 18(6):965–971.

Tiller, C.L., and C.R. O'Melia. 1993. Natural organic matter and colloidal stability: Models and measurements. *Colloids Surf.*, 73:89–102.

Tilton, R.C., J. Murphy, and J.K. Dixon. 1972. The Flocculation of algae with synthetic polymeric flocculants. *Water Res.*, 6:155–164.

Tipping, E., and D.C. Higgins. 1982. The effect of adsorbed humic substances on the colloid stability of hematite particles. *Colloids Surf.*, 5:8–92.

Yoo, R.S., W.W. Carmichael, R.C. Hoehn, and S.E. Hrudey. 1995. *Cyanobacterial (blue-green) algal toxins: A resource guide.* Denver, Colo.: AWWA.

Zabel, T. 1985. The advantages of dissolved-air flotation for water treatment. *Jour. AWWA*, 77(5):42–46.

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Members of the Organisms in Water Committee and water utility personnel who personally knew Paul Kugrens appreciated his dedication to the pursuit of basic and applied knowledge of algae, specifically the cryptomonads. His involvement with the Phycological Society of America and other professional organizations helped the

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Glossary

accessory pigments – The light-absorbing compounds found in photosynthetic organisms that work in conjunction with chlorophyll *a*, *b*, *c*, *d*, and *e* in algae.

acicular – Needle-shaped.

adjoined – In contact with each other.

aerial (aerophytic) – Living in terrestrial habitats needing only atmospheric moisture to survive.

aerotopes ("gas vacuoles") – Groups of gas vesicles; appear as brownish, refractive, irregular bodies that can be dispersed throughout the whole cell or localized (often near poles or central region); involved in the buoyancy displayed by some cyanobacteria.

akinete – A type of resting cell; larger than vegetative cells; surrounded by a thick, multilayered cell wall; important in species identification of Nostocales.

alar canal – A canal-raphe found on the edge of winglike projections of the valve (see the genus *Surirella*).

alternation of generations – The life history with two alternating phases, a typically haploid gametophyte that produces the gametes and a diploid sporophyte that forms spores by meiosis.

alveolae (= areolae) – A small cavity.

amoeboid – The unicellular life forms that mainly consist of a nucleus, cytoplasm, and sometimes contractile vacuoles (e.g., marine amoebae lack contractile vacuoles) as their basic structure and have the changeable shape of an amoeba.

antapex/antapical – The bottom of the cell and the plates that surround it.

apex/apical – The top of the cell and the plates immediately surrounding the apex.

apical axis – The longitudinal axis of a valve. The raphe or pseudoraphe lies in this axis or is eccentric to it.

apical plane – The axial plane perpendicular to the transapical axis.

archeospore – Also known as monospore; asexual spore of the red algae that germinates into the same life-history phase.

arcuate – Arched, bow-shaped, curved.

areolae (alveolae) – The polygonal or rounded pores in valve wall, often honeycomblike.

armor – The division of cellulose wall into plates that have distinctive numbers, shapes, and ornamentation.

asexual reproduction – Arise from the partitioned parent cell without the involvement of gametes.

assimilative – A stage in the life cycle when organism takes in nutrients either by photosynthesis, ingestion, or absorption.

athecate – Without cellulose plates.

aufwüchs (= periphyton) – Growing attached to a substrate, to grow upon.

autocolony – A colony formed in the asexual reproduction of a coenobium that is a miniature of the parental colony.

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autotrophic – An organism that produces complex organic compounds from simple inorganic molecules using energy from light or inorganic chemical reactions.

axial area – The clear area between raphe and ends of transverse striae.

benthic – Living on the bottom, bottom-dwelling.

biseriate – In two rows; with two series of cells.

caespitose – Clustered; in fascicles; forming a mat or tangle.

calyptra – The thickened cap on the outer cell wall of an apical cell in some filamentous cyanobacteria.

canal raphe – A raphe lying in a groove or channel, usually located in a more or less marked crest or keel.

capitate – Swollen at the ends (apices).

carotenoid – A group of organic pigments (yellow to red) found in many algae and higher plants that absorb blue light for photosynthesis and protect chlorophyll from photo-damage.

carpogonium – The female gametangium of the red algae, usually with a receptive area (trichogyne) for the male gamete (spermatium).

carposporophyte – The postfertilization stage of many red algae, usually consisting of diploid filaments that form diploid spores (carpospores)

catenate – A series of cells placed end to end in a beadlike series or chain.

central area – The clear area in the center of the valve around the central nodule.

central nodule – A thickened area between the two central pores of the raphe. See *central area.*

centric – A circular or barrel shaped diatom, i.e., *Cyclotella, Aulacoseira, Stephanodiscus.*

chantransia stage – The diploid life-history phase of members of the red algal orders Batrachospermales and Balbianiales. Forms the gametophyte directly attached to it by meiosis.

chloroplast – The organelle found in plant cells and eukaryotic algae that conduct photosynthesis.

cingulum – The transverse groove that encircles cell, usually mid-cell (like a waist), and houses the transverse flagellum.

clavate – Club-shaped.

coenobium – A colony in which the number of cells is fixed at its origin.

colonial – Varies; e.g., for cyanobacteria or green algae: a group of cells held together by a more or less conspicuous gelatinous secretion; for *Chrysosphaerella, Synura,* and *Uroglena,* a group of cells attached together in the colony center by means of cytoplasmic strands.

colony – The microscopic or macroscopic aggregations of cells or filaments.

conductivity – A measurement of dissolved ions in water. In natural waters it can be influenced by pollution. As more ions are dissolved in water, the conductivity increases.

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conjugation – The transfer of genetic material by papillae and tubes allowing direct cell-to-cell contact.

connecting bands – The ring-shaped inner part (rim) which, with the mantle, constitutes a valve. Connecting bands appear as lines in side view or *girdle.*

constricted – Pinched in.

copulae – Intercalary bands; girdle bands.

cortication – The peripheral layer typically of photosynthetic cells surrounding a colorless inner layer or filament.

costae – The ribs or thickenings appearing as dark bands on diatom cell walls.

cross-wall – The visible cell wall separating neighboring cells in filamentous cyanobacteria.

crustose – The flat body composed of compacted tires of cells; many marine algae grow closely appressed to a surface and are called crustose algae.

cuneate – Wedge-shaped.

cymbaeform – Cymbal-form; cymbal-shaped.

cytokinesis – The process whereby the cytoplasm of a single cell is divided to spawn two daughter cells.

dentate – Toothed; notched.

diffluent – A surface that will melt away as opposed to a hard sheath dissolving away.

diploid – Cells that have two homologous copies of each chromosome, or twice the number of basic chromosomes.

discoid – Like a disc or shortened cylinder.

elliptical – Oval-shaped.

endogloeic – Living in the mucilage of other algae.

epilithic – Living on the surface of rock.

epipelic – Living on the surface of sediment.

epiphytic – Living attached to plants or other algae.

epipsammic – Living attached to sand grains.

epitheca – The upper portion of a cell, above the cingulum.

epivalve – The upper, and therefore older, half of a frustule, which fits closely over the *hypovalve.*

epizooic – Living attached to animals.

euplankton – Planktonic organisms that are entirely free-floating or found in open water habitats.

exocyte ("exospore") – The reproductive cells produced by budding from another cell.

false-branching – The branching formed by lateral growth of one or both ends of a broken trichome; appearance of branched cells but when cells are simply adjacent to each other and connected only by mucilage.

fascicle – A bundle or cluster of filaments.

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filament – A row of cells joined end to end, or side by side, either adjoined or held together in line by gelatinous threads.

flagellum – A long, slender projection from the cell body used in motility, which can be directly seen or rendered visible after appropriate treatment with the light or electron microscope.

floridean starch – The carbohydrate storage granules in the cytoplasm of red algal cells; a branched glucose polymer.

frustule – The cell wall (shell) of a diatom composed of two valves joined by a *connecting band* known as a *girdle*.

fucoxanthin – A brown carotenoid pigment common in diatoms and brown algae.

fusiform – Torpedo-shaped or spindle-shaped; a shape wide in the middle and tapering at both ends.

gametangia – The organs or cells in which gametes are produced.

gamete – A cell that fuses with another compatible cell to form a zygote.

gametophyte – The gamete-producing phase of an organism that exhibits an alternation of generations.

gas vesicles – The small intracellular vesicles, aggregated to form aerotopes; involved in the buoyancy displayed by some cyanobacteria.

girdle – The bands of silica attached to each valve mantle, one girdle overlapping the other. See *connecting bands*, *girdle bands*.

girdle bands – The ring-shaped inner part (rim) which, with the mantle, constitutes a valve. Connecting bands appear as lines in side view or *girdle view*. See *girdle*.

girdle view – The side view, with the overlapping halves (*valves*) of the *frustule* apparent, the *girdle* uppermost in optical view.

granule – The inclusions within cells that are visible by light microscopy; often used for storage; can be distributed throughout cells or concentrated near cross-walls or cell center.

haplontic – Life cycles in which there is no multicellular diploid phase.

heterocyte ("heterocyst") – The special cells present in some cyanobacteria that are involved with nitrogen fixation; they are thick walled, usually pale-colored, and often with cyanophycin granules at the side(s) that are in contact with vegetative cells.

heteropolar – The ends of the valve that are different (*hetero*) from one another. Describes filaments, trichomes, valves, and thalli where the opposite ends have different morphologies (valve ex. *Gomphonema* sp.).

holdfast cells – The cells that act as a rootlike structure that anchors algae to the substrate.

holoplankton – The plankontic organisms that spend their entire life cycle within the water column.

hormocyte – An ensheathed hormogonia.

hormogonia – The short pieces of cyanobacteria trichomes that separate from the original trichome after fragmentation or formation of necridic cells; act as a mode of reproduction.

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horn – A much elongated process that is not noticeably tapered (cf. *spine*).

hyaline area (= pseudoraphe) – The area that appears to be colorless and or featureless on the valve face.

hypnozygote – A thick-walled zygote that lays dormant until optimal conditions arise.

hypotheca – The lower portion of a cell, below the cingulum.

hypovalve (= hypotheca) – The lower (and younger) valve.

intercalary – The plates between apical and precingular series (apical intercalary), or between antapex and postcingular series (posterior intercalary).

intercalary bands – The bands of silica often occurring between the *valve mantle* and the *girdle*. These are variable in number and width and sometimes extend into the valve to form a *septum*.

isodiametric – Having equal diameters.

isopolar – The ends that are not different from one another; they have identical morphologies.

keel – A projection of the valve surface, as a keel of a boat, usually more or less eccentric to the *apical axis*. In it is enclosed the *canal raphe* that is characteristic of certain genera.

keel punctae – The pores, or membranes appearing as pores, in the plate lying below the *canal raphe*. These "carinal dots" (carinal = canal) are quite conspicuous and characterize a few genera.

kleptoplastids – The chloroplasts acquired from a photosynthetic alga and retained and used by a new host.

lamellate, lamellated – Describes colonial mucilage or mucilaginous sheaths with layers or that is layered.

lanceolate – Lance-shaped; long and narrow with margins tapered at the apex.

linear – Long, straight, and narrow.

lineolate – Striae that appear to be striped (with lines).

longitudinal – Along the long axis (cf. *transverse*).

longitudinal axis – Axis running along the length of the thallus.

lunate – Crescent-shaped.

macroscopic – A term used to describe physical objects that are measurable and observable by the naked eye.

mantle (= valve mantle) – The outermost part of the valve that is apparent in girdle view, but excluding the connecting band; the broad surface as seen in valve view.

mat – The macroscopic layer of filamentous algae covering some substrate (usually submersed or on wet soil).

medulla – The inner layer of a differentiated thallus that is usually colorless; surrounded by an outer photosynthetic layer (cortex).

meroplankton – The plankontic organisms that spend only part of their life cycle within the water column before settling on the bottom, becoming benthic.

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metaphytic, metaphyton – Algae that are normally loosely associated with (not attached to) aquatic plants; often occurring in the littoral zone of lakes, ponds, and wide rivers.

microscopic – A term used to describe physical objects that must be observed with a microscope.

mitosis – The process by which a cell duplicates the chromosomes in its cell nucleus in order to generate two identical daughter nuclei.

monospore – See *archeospore*.

monostromatic – Single layered.

motile – With the ability to move actively.

mucilage – A layer of complex polysaccharides surrounding individual cells or entire colonies.

multiaxial filament – See *multiseriate filament*.

multiseriate – Composed of more than one row of cells.

multiseriate filament – Also known as multiaxial filament; the main axis with multiple parallel filaments.

naked – Without cellulose plates.

nanocytes – Small reproductive cells, differentiating from mother cells after fission within fine, gelatinous slime.

navicular – Boatlike; boat-shaped.

necridic cells (necridia) – Dead cells that function to aid fragmentation of trichomes prior to formation of hormogonia or false-branching.

nodule – A small knob; internal wall thickening located centrally or at the poles; thus either *central nodules* or *polar nodules* between which lie the raphe.

ocellus – A raised, rounded, or ovate thickening of the valve surface, having the appearance of a large pore or eyelike spot.

oogonium – The female gamaetangium that produces an egg or eggs.

oval – Elongate and rounded with curvature the same at both poles.

papilla – A protuberance or swelling.

pennate – A diatom *frustule* shaped like a pen, or penlike; elliptical.

pericentral cell – The cell formed immediately adjacent to the main axis of a filament.

periphytic – On top of something in aquatic systems (*aufwüchs*).

pervalvar axis – A line connecting the middle point of the two valves.

phycobiliproteins – The water-soluble pigments of the cyanobacteria, red algae, and cryptomonads that are red or blue-colored.

pigments – The organic chemical that changes the color of light it reflects as the result of selective color absorption and captures the light energy necessary for photosynthesis.

planktonic – Free-floating.

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plastids – The major organelles found in algae that contain photosynthetic chemicals such as chlorophyll a, accessory pigments and other components of the photosystem.

polar nodule – An enlarged, usually thickened area of the wall in which the raphe terminates, forming an external and an internal fissure.

postcingular – The plates below the cingulum in armored taxa.

precingular – The plates above the cingulum in armored taxa.

process – Any structure protruding from or projecting; any extension of a cell such as a spine or horn.

pseudofilament – A loose chain of cells held together in a common mucilage but cells do not share common cross-walls.

pseudoparenchyma(tous) – The thallus that resembles a mound of cells (parenchyma) but is actually constructed of closely growing filaments.

pseudoraphe – A clear, axial line devoid of wall markings or of a raphe; a longitudinal, clear space laterally separating the two vertical series of transverse striae, either median in position or eccentric.

puncta (punctae) – Minute pores or dots; valve markings usually in rows (cf. *striae*).

pyrenoid – The center of carbon dioxide fixation within a differentiated region of a chloroplast were starch formation occurs.

quadrate – Square or approximately so.

raphe – An axial, groovelike slit in the main axis of the valve. Characteristic only of motile pennate genera.

red tide – Large numbers of algae that discolor the water.

reticulate – Netlike.

rhombic – In the form of a rhombus, which is a parallelogram having its angles oblique.

rostrate – Beaklike. Usually used for the shape of the ends of valves.

septa – Incomplete partitions running parallel with the valves that result from internal extensions of the intercalary bands. They may extend completely across the valve or may be only developed at the margin.

sessile – Attached to the substrate by gelatinous secretions.

seta – A slender, bristlelike organ or part; a long thin spine.

sexual reproduction – The reproduction involving the paired union of special gametes from two individuals.

sheath – The morphologically distinct layers of mucilage external to cell walls; can be firm, thin, or thick and colorless or colored; can be open or closed at the ends of filaments; and can be lamellated or not.

sigmoid – S-shaped (more or less), the apices curving in opposite directions.

spermatangium – A male gametangium in the red algae that is typically colorless and produced at the tips of vegetative branches; forms the male gamete, the spermatium.

spermatium – A male gamete in the red algae that is colorless and without flagella for locomotion.

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spine – An elongate process tapering to a blunt or sharp tip.

stauros (Gr. staur, cross) – A central nodule that extends almost to the margins of the valve, is thickened and expanded, and with a clear axial area that looks like a cross.

striae – Delicate, long, linelike markings usually occurring in parallel rows; lines of punctae so crowded as to appear as a solid line.

sub- – A prefix denoting a lesser degree of the term with which it is combined.

subaerial (subaerophytic) – Living in terrestrial habitats that receive intermittent moisture, such as dripping water.

suborbicular – Nearly, but not quite, circular or spherical.

sulcus – A longitudinal groove on the ventral face of the cell that houses the longitudinal flagellum. The sulcus usually extends to the antapex from the cingulum and may extend slightly into the epitheca.

terminal nodule (= polar nodule).

thallus (thalli) – The general form or body of an alga; generally referring to macroscopic morphology or characteristics.

theca/thecate – A cell that has cellulose walls divided into plates.

thylakoid – A membrane-bound compartment inside chloroplasts that contain photosynthetic pigments.

transapical axis – The transverse axis of the valve.

transapical plane – An axial plane perpendicular to the *apical axis*.

transapical view (= narrow girdle view) – The view with apical axis turned toward observer.

transverse – Across the short dimension.

transverse axis (= transapical axis) – The axis of the valve that connects the two margins of the valve and is perpendicular to the apical axis.

trichogyne – The receptive portion of the female gametangium (carpogonium) found in certain green and red algae.

trichome – Certain algae that have a row of cells that are connected into one physiological unit (elongate hairlike structure or "filament").

true branching – The branching that follows lateral division of cells resulting in cells growing more or less perpendicularly to the original primary axis of a trichome; branches are physiologically connected to the original trichome.

truncate – Having the ends square or even.

tumid – Protuberant.

tychoplankton – The algae that become resuspended by factors like turbulence into the water column and that are typically not found in planktonic communities. This includes most benthic diatoms.

ultrastructure – The detailed structure of a biological feature or specimen (e.g., scale, flagellum, cell, tissue) observed using an electron microscope.

undulate – Wavy in outline.

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uniaxial filament – See *uniseriate filament.*

uniseriate – The cells arranged in a single series or row.

uniseriate filament – Also known as uniaxial filament with a single chain of cells, either branched or not.

valvar plane – An axial plane perpendicular to the pervalvar axis.

valve – One of two overlapping halves of a frustule composed of a flattened or undulate surface and a mantle that is perpendicular to it (see *epivalve* and *hypovalve*).

valve mantle – The portion of the valve that is apparent in *girdle view* (see *mantle*).

valve view – The view in pennate diatoms from the top or bottom so that the broader, flatter surface of the valve is seen, the valve side uppermost; the view one sees looking upon the surface of the valve. In centric forms, the more or less circular (or angular) end view.

vegetative cells – Cells not specialized for a specific function (such as reproduction, nitrogen fixation).

wing – A thin projection of the valve surface, much better developed than a keel, often arising near the apical axis of the valve but becoming most apparent at the junction of the valve with the valve mantle, where the surface of the valve may be distinctly elevate. The canal raphe may be enclosed in the wing.

zoospore – A motile asexual spore utilizing a flagellum for locomotion.

zygote – A cell that is the result of genetic union.

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